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UNITED STATES DEPARTMENT OF AGRICULTURE

Artifical Insemination in Livestock Breeding[1]

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CONTENTS

[1] This revision supersedes earlier editions by W. V. Lambert, formerly in the United States Department of Agriculture, and Fred F. McKenzie, formerly at the Agricultural Experiment Station, University of Missouri, and agent in animal husbandry, United States Department of Agriculture.

INTRODUCTION

In recent years, a great interest has arisen in artificial insemination of livestock, and much progress has been made in its application. Artificial insemination refers to the artificial introduction of semen into the genital tract of the female, as contrasted with natural insemination, in which semen is introduced into the female genital tract by the male.

Insemination may occur without a resulting fertilization and pregnancy. Fertilization refers to the union of the male reproductive cell, or spermatozoon, with the reproductive cell, or egg, of the female. It is possible that fertilization may occur and the resulting fertilized egg may not become implanted (attached to the mother's uterus), in which case pregnancy is terminated.

Artificial insemination is being used as a tool for improving livestock in nearly every country of the world. It is used most extensively in dairy-cattle breeding and, in some countries, on a wide scale in sheep breeding. Techniques have been developed for its use in horses, swine, dogs, rabbits, poultry, and fur animals.

Dairy-herd-improvement records have shown that a few bulls have outstanding ability to sire daughters with higher milk and butterfat yields than those of their dams. Artificial breeding offers a means of obtaining many times as many daughters as can be obtained by natural service. Furthermore, most bulls submit to the techniques used for collecting semen, and the semen retains its fertility both after high dilution and for a sufficient time to allow its transportation and use over wide areas. The dairy-cattle population is concentrated to the degree that large numbers of animals are available for insemination within a comparatively small area.

HISTORICAL ASPECTS[2]

Although the application of artificial insemination is comparatively new, the concept is old. There is evidence that the method was known and perhaps used by Arab horse breeders about 1300 A. D. However, the first authentic account of its use in mammals was by an Italian, Spallanzani, who successfully inseminated a bitch about 1780. This result was confirmed in 1782 by Pierre Rossi, who took the special precaution of keeping the bitch under lock and key throughout the entire heat period.

From that time until the latter part of the nineteenth century apparently few attempts were made to use artificial insemination, although occasional references are made in both medical and veterinary literature to its practice as a means of overcoming sterility. Between 1884 and 1896, Heape states, Sir Everett Millais carefully repeated

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[2] These brief notes on the historical aspects of artificial insemination were taken largely from the papers of Heape (*33*)[3] and Iwanoff (*35*). For a detailed account of the historical aspects of this subject the reader is referred especially to the latter paper.

[3] Italic numbers in parentheses refer to Literature Cited, p. 67.

Spallanzani's experiments. Of a total of 19 bitches inseminated, 15 conceived. In the early 1900's the practical potentialities of artificial insemination began to be recognized, and in 1907 a Russian physiologist, Iwanoff (*35*), reported the results of a series of inseminations in mammals. These experiments were so successful, according to Walton (*85*), that a laboratory was established in 1909 for the purpose of training veterinarians in the technique of artificial insemination.

These studies were interrupted during World War I, but shortly after its close experiments were again undertaken on a large scale and with such practical results that, according to the Russian investigator Keršin (*36*), more than 6 million cattle and sheep were artificially inseminated in the Soviet Union in 1936. In England, the United States, and elsewhere many investigations also were undertaken after the war. The number of workers in this field has increased rapidly, and a vast literature has accumulated concerning this subject. The reader is referred particularly to the work of (1) Bonadonna and Bovati (*15*), which gives a summary in Italian and English of books and publications related to artificial insemination in Italy from 1914 through 1945; (2) of Bonadonna (*14*), whose publication covers the field on the collection of semen and the technique of insemination, which is well illustrated; and (3) of Perry and associates (*58*), who discuss artificial insemination of farm animals. Each contributor in the last-mentioned work writes on the subject for which he is best qualified.

Following the introduction of artificial insemination into most countries, animal improvement has been so gratifying that use of the method has rapidly increased, as shown by accounts given at the First International Congress on Physiology and Pathology of Animal Reproduction and of Artificial Insemination, held at Milan, Italy, in 1948 (*21*). Methods of collecting semen, handling it from time of collection to insemination, and effecting insemination have been improved. Hand in hand with these developments, many facts have been established concerning the biology of sperm cells, the secretions of the glands of the reproductive tract of the male, and of estrus and its related phenomena in the female. Two of the more recent developments that have greatly increased the use of valuable sires are the successful shipping of the semen over long distances and maintenance of the original fertility of the semen for use in greater dilutions.

ADVANTAGES AND LIMITATIONS OF ARTIFICIAL INSEMINATION

Making Better Use of Young Sires

In young sires, the number of services ordinarily obtained is limited. Consequently, by diluting their semen, which is one of the practices in artificial insemination, and distributing its use among a comparatively large number of females, an adequate number of the latter animals can be inseminated to prove the breeding ability of these sires much earlier than through natural service. For instance,

rams ordinarily are not used for breeding until they are 15 to 20 months old. If by the use of artificial insemination they can be used for breeding as lambs, 1 year will be gained in the age at which they are proved. Likewise, young bulls and stallions can be proved at younger ages than by natural mating.

Increasing the Use of Valuable Proved Sires

The reproductive life of sires in most species of livestock is short, and several years at best must elapse before a sire can be tested adequately for such a character as milk production of his progeny. It is important, therefore, that the fullest use be made of proved sires. Artificial insemination is an effective means of doing this through transportation of their semen over great distances and by diluting it for use in large numbers of females.

Experiments have shown that semen can be shipped great distances and still retain its fertilizing qualities. It is common practice in the United States to transport semen of proved bulls to any location within the country supplied by regular air schedules. In artificial-breeding associations, semen is commonly diluted 1 to 30 or more times for regular use and it has been diluted more than 1 to 100 times for experimental purposes without loss of fertility. In some associations exceptionally good bulls are each siring more than 2,000 offspring per year.

Spreading the use of valuable sires can be done very effectively in many breeding associations, particularly in view of the improvement in techniques during the last few years. Within recent years a number of cooperative dairy breeding associations have been organized in the United States, the main purpose of which is to obtain for the members of the association the service of proved sires at a reasonable cost. In 1940 there were 27 cooperative dairy breeding associations, located in 16 States, inseminating an estimated 33,000 cows. By January 1, 1949, the number had increased to 1,263 associations in 47 States, with an enrollment of 2,412,160 cows, or about 10 percent of the dairy-cow population. Some States have a limited service, usually confined to a few counties, whereas some Eastern and Midwestern States have nearly a complete coverage. Individual associations have increased in size. The largest breeding non-affiliated cooperative inseminated 108,000 cows in 24 counties in 1948, as compared with only 5,000 cows in 5 counties in 1941.

Information about the organization of such associations may be obtained by writing to the Director of Information and Extension, Farm Credit Administration, Washington 25, D. C., for a copy of Circular C–133, Dairy Breeding Cooperatives: Their Development, Practices, and Policies.

Because of age or crippled condition, a male may be unable to mate normally or, if capable of completing the act, may for similar reasons tire readily and be able to serve only a small number of females. Artificial insemination may extend the period of usefulness of such sires.

In species in which the males are largely monogamous, artificial insemination also offers promise of increasing the use of valuable sires, which would be a real boon to breeding progress in such species.

Increasing the Percentage of Conceptions

Asdell (*4*) says that in most species the maximum fertilizing ability is reached when insemination is performed early enough for spermatozoa to reach the top of the oviduct at the time of ovulation. This suggests a short life of the egg. Fertility of the spermatozoa is reduced after a stay of 24 hours or longer in the female tract. Various studies on the rate of travel of spermatozoa indicate that they reach the oviduct in less than 1 hour to 6 hours after insemination. Thus, the possibilities of fertilization are increased when the female is inseminated a few hours before ovulation.

In horses, for which the percentage of conceptions probably does not exceed 50 to 60,[4] the percentage might be increased if mares were served more than once during heat. Heat periods in mares range from 4 to 30 days, with an average of 5 days. Ovulation normally occurs 20 to 40 hours before heat ends, and theoretically the best time to mate would be a few hours before ovulation. Artificial insemination may be effective in bringing about insemination when the chances for conception are greatest and when service by the stallion is not available. To this end, therefore, it should be performed every other day until the end of heat.

In cows ovulation varies from 1 to 30 hours after cessation of heat, with an average of 14 hours. Cows habitually ovulating late could be artificially inseminated after cessation of heat, at which time they refuse to stand for the bull.

Overcoming Difficulties Due to Difference in Size and Other Causes

If natural service is impossible because of marked difference in size or weight, artificial insemination may effect conception. This statement applies especially in the breeding of young females to old sires.

Artificial insemination has been used in a few cases to produce certain racial and species crosses when, owing to differences in size, anatomical structure, or psychological characteristics, natural mating has been impracticable. In this way it has proved useful as a tool for hybridization experiments.

Limitations of Artificial Insemination

Although artificial insemination is a promising procedure for the breeder, it can be used successfully and with safety only by skilled persons specially trained. It is necessary to know the structure of the reproductive organs and the procedures in collecting the semen and introducing it into the genital tract of the female. Spermatozoa are very delicate and must be handled with great care if they are to retain their full vitality, which is necessary for the highest percentage of successful impregnations. Care needs to be taken also to avoid

[4] Prof. T. A. Ewing, University of Missouri, in a survey of breeding records in Missouri found that of 2,895 mares bred, 1,608 (55 percent) settled and 1,323 (45 percent) produced foals.

injury to the reproductive system of both male and female and to prevent the possible spread of disease.

Artificial insemination does not surpass natural breeding in percentage of pregnancies per service, but when semen from fertile males is used properly it may nearly equal it. Frequently owners wish to use it to cure difficult breeding conditions and become discouraged because of its failure to induce pregnancy from the first few inseminations. Such practices should be discouraged.

CONDITIONING THE MALE FOR BREEDING

An important consideration in all breeding programs, whether natural breeding or artificial insemination is practiced, is to keep the male in the best possible breeding condition. The condition is described by the English investigators, Marshall and Hammond (*46*), as a "hard one produced by sufficient exercise to work off a surplus of fat, but favouring the retention of nitrogenous substances and vitamins." Management should be such that breeding males and females should be improving in condition throughout the breeding season, for an improving condition is more favorable to the normal functioning of the reproductive system than a stationary or declining condition. Excessive fatness in breeding males should be avoided. Although such males may not be sterile, their fertility is lowered by the production of either fewer or lower quality spermatozoa.

The ration of the male should be properly balanced and contain ample supplies of proteins, minerals, carbohydrates, and vitamins. The constituents of a ration will vary from region to region and to some extent with price changes in various feedstuffs.[5] When possible, the male should receive green feed for several weeks before and during the breeding season, and he should have access to good water at all times.

Males receiving an adequate amount of exercise will produce, on an average, larger ejaculates containing a greater number and a higher quality of spermatozoa than males not properly exercised. They will also be more active and will deliver more services in a given interval of time. Care should be taken to avoid overheating, especially in warm weather. Turning bulls together in a large enclosure should provide ample exercise.

Where summer temperatures are frequently above 80° to 85° F. it is good practice to allow the male access to cool quarters, cool water for drinking, and ample shade. It is important to avoid excessively high temperatures throughout the summer. Unpublished work done at the Missouri Agricultural Experiment Station indicates that Shropshire and Hampshire rams protected from temperatures above 80° produced more semen and a larger number of normal spermatozoa and were prepared to enter the breeding season earlier than similar rams subjected to the usual temperatures of 80° to 100°.

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[5] The reader is referred to publications on feed in relation to spermatogenesis by Branton and associates (*17*) and Reid (*67*) and to the U. S. Department of Agriculture and State experiment stations or to Morrison (*54*) for information pertaining to the feeding of livestock.

CARE OF EQUIPMENT AND PROPER QUARTERS FOR BREEDING ANIMALS

In the practice of artificial insemination, regardless of the species, there are certain procedures that must be followed as well as certain precautions that must be taken to insure the greatest success. Three distinct operations are involved—collection and examination of semen from the male and insemination of the female. The techniques of collecting semen vary somewhat with the different species; consequently, they are presented separately for each species (pages 33 to 63). However, if artificial insemination is to be employed to any extent in a breeding program, other factors involved in the collection of semen must be considered. Among these are the care of the apparatus used and proper quarters for the breeding animals.

Before collecting semen or inseminating the female, it is necessary that the proper apparatus be on hand and that it be clean, free from harmful chemical substances and bacteria, and completely dry. Special attention should be given to the cleanliness of the artificial vagina. It may be cleaned satisfactorily by taking it apart and washing the rubber pieces with soap or a detergent and water. After a thorough rinsing to remove all traces of soap or detergent, it may be sterilized by either boiling or rinsing in ethyl alcohol—never methyl or denatured alcohol. Boiling is preferable, but it may hasten deterioration of the rubber. For sterilizing with alcohol, the vagina is assembled and thoroughly rinsed with 65- or 70-percent grain alcohol. After it is emptied, a sterile plug of cotton may be inserted into the open end and the vagina stood on this end with the collecting tube secured above to allow complete drainage and drying. The least trace of alcohol is harmful to spermatozoa. If used before 24 hours after rinsing, the apparatus should be rinsed with physiological saline. Several rinses are necessary to remove all traces of the alcohol.

Shortly before use, the instruments should be placed on a clean towel or blotting paper on a solid but movable table close to the operator, and they should be covered with a clean towel to keep them free of dust. The operator should wash his hands thoroughly with soap and hot water, then rinse and dry them. Since the equipment needed varies somewhat for the different species, it is discussed separately for each species.

The paddock or quarters should be ample in size, level, and free from objects that might frighten or injure breeding animals. For the larger animals, smooth concrete floors should not be used because of the danger to the animals from slipping, especially when the floor becomes wet with urine. If possible, the same paddock or breeding quarters should be used each time, since males serve best in familiar surrounding and they soon learn to anticipate service when led into these quarters. Psychic factors may influence the action of some males (page 39). For both collection and insemination, the crates or other equipment should be so arranged as to be most convenient for the operator.

TRAINING THE MALES

Another factor involved in the collection of semen is the training of the males. When males are to be used extensively for artificial

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insemination, it is very important that they be docile, well broken to lead, and free from bad habits. Most large artificial-insemination studs have facilities for the safe handling of aggressive bulls.

The male should be trained to mount the female without undue delay, although too rapid mounting should be discouraged for there is evidence that a reasonable amount of time expended by the male in maneuvering previous to service results in a larger quantity of semen and better quality of spermatozoa. McKenzie and Berliner (*43*) have shown that in rams with a strong sexual impulse and that mounted ewes quickly, the number of spermatozoa was less in the first ejaculate than in subsequent ejaculates. One of the most important factors in the preparation of a bull for collection is to hinder him from ejaculation until the accessory gland secretions have been discharged. The result will be a greater quantity and better quality of semen.

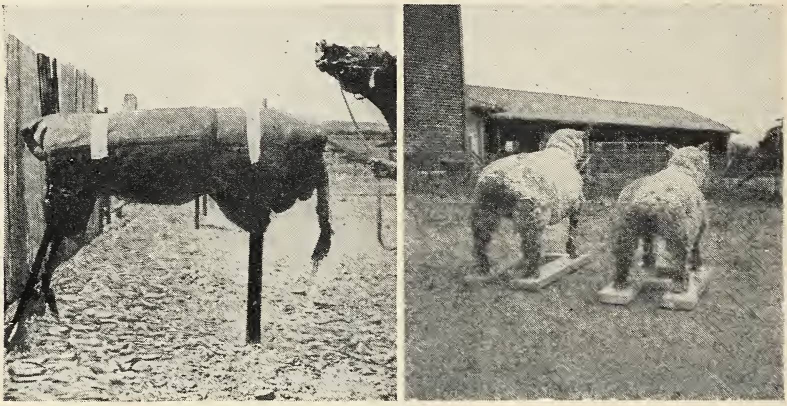

FIGURE 1.—Dummies used for the collection of semen at the animal husbandry experiment stations at Miles City, Mont., and Beltsville, Md. Note that two sizes of sheep dummies are used to accommodate different sizes of rams. The framework of each dummy is of metal, which is either bolted to the platform or firmly implanted in the ground as shown at left. Note that ample space is provided under sheep dummies for insertion of the artificial vagina, which is held in place by rubber bands or by the operator.

If the artificial vagina is used for the collection of semen and frequent collections are to be made, the use of dummies is practicable. It eliminates the necessity for having females in heat and also the extra work involved in having females on hand for collection.

The males of most species can be readily trained to use dummies. Sokolova (*76*) found that boars without exception, but not all stallions and bulls and only a few rams, use dummies readily. However, the use of dummies is not to be recommended for any species unless a large number of collections are to be made with the artificial vagina. In training the male to use a dummy he should first be used to serve females that are in heat, and the same quarters should be used for this purpose each time. As a result he soon learns to anticipate service when brought into these quarters, and after he has become accustomed to expecting service the dummy may be substituted for the female. Not all males train with the same ease, and with some species more difficulty is encountered than with others. Young, vigorous

males as a rule may be most readily trained to use the dummy. Sight or odor apparently plays a relatively small part in the mating instinct of the male in most species although some variability in this respect exists between individual males as well as between males of different species. In the boar, sexual attraction plays little part in the mating behavior as this animal is easily stimulated to attempt mating by the presence of a dummy sow.

The dummy should be solidly constructed and firmly anchored and should resemble somewhat, although not necessarily, the female of the species for which it is being used (fig. 1). The framework may be of either metal or wood, the only precaution necessary being that it be adequate to support the weight of any males used for collection. The top and sides of the dummy should be well padded and the structure covered with a skin, canvas, or other durable material. Space may be provided for the insertion of the artificial vagina. The latter may be held in place with straps, but in the larger models it is better if it can be held by an assistant, who should be seated under the dummy.

FREQUENCY OF SERVICE AND REPRODUCTIVE CAPACITY

Great variation in the rate of exhaustion of spermatozoa in successive ejaculates exists between males of the different species and even of the same species. Much variation also exists in the rate of replenishment of the sperm supply following periods of rest after service, according to McKenzie and Berliner (*43*). For those species that produce a large volume of ejaculate at each mating, such as the horse, boar, and dog, the number of spermatozoa per ejaculate, as well as their viability, decreases rapidly. For other species, such as the bull and ram, which ejaculate relatively small quantities of semen at one time, many matings may be made in a relatively short time without greatly decreasing the number of spermatozoa per ejaculate or their viability.

With many mature stallions, two to five matings may be made on some days without lowering the average level of fertility. Lighter use, however, is best following days with heavy breeding schedules. According to Polowzow (*63*), a rest period of 24 hours is sufficient to restore the number of spermatozoa to normal and to eliminate the high proportion of nonviable spermatozoa that are evident after frequent matings.

In boars, whose volume of semen per ejaculate is the largest among domesticated animals, there should not be more than one mating a day, according to Rodolfo (*70*), and even then there should be an interval for rest after every 2 days. This is particularly true for yearling boars, which probably should not be used oftener than once every other day if they are to be used as long as 2 weeks; otherwise the number of spermatozoa decreases markedly, and immature spermatozoa begin to predominate, as explained by McKenzie, Miller, and Bauguess (*44*). In farm practice boars are used much more frequently. They are generally pasture-bred to 15 to 20 sows each, not more than 1 or 2 sows needing a second service.

With bulls and rams, many successive matings may be made in 1 day without depleting the sperm supply, as shown by McKenzie and Berliner (*43*). Bulls in Denmark are used regularly three times a

week, and in many instances daily collections have been made without impairment of fertility. However, collection of semen from bulls in the United States is generally limited to once a week or every 10 days. Consequently, in a bull stud in which semen samples are taken three times weekly, a minimum of five or six bulls is needed for each breed. When six or seven samples a week are to be taken, eight bulls are believed to be the minimum number needed. A proportionately smaller number of bulls is needed when daily ejaculates are taken, since higher dilutions are possible because semen is not stored so long. In rams, more than three matings a day can be made without serious depletion of spermatozoa. Although the number of spermatozoa decreases in each ejaculate with successive matings, the sperm supply never seems to be permanently exhausted through successive matings, and a short rest period seems to suffice for the production of a fresh supply of sperm. Much variation is observed between individual rams in these respects, and there is also seasonal variability in the rate of sperm production.

Sexual desire is not a reliable index of sperm-producing capacity of males. For instance, in sterile males or in double cryptorchid males in which the spermatogenic function is absent, the mating desire may be very high, and good rams may continue to copulate when the supply of spermatozoa has been temporarily exhausted by frequent matings. However, in those species which have a more or less well-defined breeding season it has been shown by McKenzie and Berliner (*43*) that more copulations occur during periods of high fertility and that there is a higher rate of sperm production during such periods.

MACROSCOPIC EXAMINATION OF SEMEN

Great variation exists in the quantity and quality of semen obtained from males of the different species (table 1), from males of the same species, and even from the same male at different times. As the number of inseminations that can be made from any one male depends on the volume of semen produced, it is desirable to use males producing large quantities of semen containing many active, viable spermatozoa. Consequently the semen of any male should be carefully examined before it is used for insemination.

Volume of semen may be measured in a graduated test tube, bottle, or pipette. Although a large volume does not necessarily mean a high spermatozoa count, for most animals there is a reasonably high correlation between the total quantity of semen in the ejaculate and the number of spermatozoa contained therein, provided, of course, the ejaculate is complete. The quantity and quality of semen may be affected by the method of handling the male at the time of collection (p. 8 and 39). Also, a seasonal variation is likely for all species of animals, especially those having a more or less limited natural breeding season. Measurement of the volume of semen for any given sire should not be made on one ejaculate but on all the ejaculates produced in a given interval of time on several occasions.

TABLE 1.—*Some quantitative characteristics of semen from various animals* [1]

Animal	Volume per ejaculate		Sperm concentration (per cubic millimeter)		Hydrogen-ion concentration	Volume of diluted or undiluted semen recommended per artificial insemination [2]
	Approximate range	Most common volume	Range	Most common		
	Cubic centimeters	*Cubic centimeters*	*Number*	*Number*	*pH*	*Cubic centimeters*
Stallion	40–320	75–150	30, 000–800, 000	60, 000	7. 0–7. 8	10. 0–30. 0
Bull	0. 5–14. 0	3. 0–4. 0	300, 000–2, 000, 000	800, 000	6. 5–7. 5	. 5–1. 5
Ram	. 5–2. 0	. 8	500, 000–6, 000, 000	1, 000, 000	6. 2–6. 8	. 1–0. 2
Boar	125–500	200. 0	25, 000–1, 000, 000	100, 000	6. 8–7. 2	50. 0–100. 0
Dog	2–19	7. 0	1, 000, 000–9, 000, 000			
Fox	. 1–4. 5	1. 5			6. 2–6. 4	
Buck (rabbit)	. 4–6. 5	0. 7	100, 000–2, 000, 000	700, 000	6. 8–7. 5	. 25–1. 0
Turkey tom	. 1–0. 7	. 3				. 05
Cock	. 1–1. 5	. 6	50, 000–6, 000, 000		7. 3–7. 8	[3]. 1

[1] No data are available for the goat.
[2] The volume here recommended is for cervical insemination. In birds the semen is introduced directly into the oviduct.
[3] Inseminations should be made at least at weekly intervals, preferably every 3 to 4 days.

The color and consistency of semen vary with the different species. They also give some clue as to the quality of the semen. A yellowish color may indicate the presence of pus or urine, which is often detected also by smell. A pinkish or reddish color indicates an admixture of fresh blood, whereas a deeper red or brownish color probably indicates the presence of degenerating blood and tissues. Such abnormal color of the semen may be associated with abnormal conditions in the genital organs of the male or possibly of the female if the sample was collected directly from the vagina. A sire from which abnormal semen is collected should not be used for insemination or breeding purposes until the cause of the abnormalities has been established and removed. If semen is collected from the vagina, care should be used to see that the female is healthy. Because of admixture with vaginal secretions, semen so collected will have a somewhat different appearance from that collected in other ways.

MICROSCOPIC EXAMINATION OF SEMEN

Motility of Spermatozoa

Motility of spermatozoa, although it has its limitations, is the most used criterion for evaluating semen quality. However, the method of collection greatly affects motility. Because the life of spermatozoa in the vagina is relatively short, vaginal samples are seldom suitable for direct microscopic examination for viability. Most reliable results are obtained from samples collected in the artificial vagina.

Samples for examination should be taken immediately after collection and should be representative of the whole ejaculate. Certain portions of the ejaculate may have a low concentration, especially in the stallion, jack, boar, and dog, in which the secretions of the accessory reproductive glands make up a large part of it.

Artificial insemination associations use the motility test to determine the quality of each sample before releasing it for insemination purposes. This simple test can be quickly employed before the semen is rushed to the inseminator. A drop of freshly collected semen is placed on a slide kept near body temperature and examined under low power. Experience soon enables the examiner to estimate the percentage of viable spermatozoa producing the swirling currents.

Blom (*10*) describes a simple technique for estimating motility. To make the results more consistent and uniform he had a special slide designed. The slide is provided with a cover slip which fits over three chambers, each of a different depth to provide for examination of swirly movements, concentration, and movement of individual sperm.

Swanson and Herman (*81*) rated motility of semen samples according to the vigor of the spermatozoa. No motility was rated as 0; less than 40 to 50 percent of spermatozoa in motion as 1; progressive degrees as 2, 3, and 4; and the best motility as 5. They state that the property of semen most nearly correlated with fertility was the length of time that the spermatozoa remained in a vigorous condition at a storage temperature of 4° C. Those bulls whose semen maintained good motility for less than 24 hours, on the average, were of poor fertility. They propose that a bull's fertility should be rated from

examinations of at least five semen samples collected during a period of at least 2 weeks.

In a study of bovine spermatozoa Rao and Hart (*64*) classify motility into three types—maximal, circular, and convulsive—depending on the vigor and direction of movement. Maximal motility is the most active type, in which the movement is in a straight line and the sperm head assumes an on-edge position. Circular motility is exhibited by spermatozoa with abnormalities of the tail but evidencing considerable vigor and endurance. Convulsive motility indicates decline in vigor and ranges from practically no movement except lashing of the tails to a fairly active serpentine movement.

These investigators use a system of five grades to express the intensity of motility. The semen is examined under a magnification of 250, using a warm stage set at 37.8° C. Good indicates that about 80 percent of the spermatozoa are alive and most of them are in maximal motility; medium, about 65 percent alive and a large portion in maximal motility; fair, about 50 percent alive and only a small portion in maximal motility; poor, about 35 percent alive and few or none in maximal motility; dead, no movement. The percentage of live spermatozoa was ascertained with the opal blue-eosin staining mixture reported by Lasley and coworkers (*40*). Rao and Hart state that motility has been criticized as an inadequate index of the fertilizing capacity of the spermatozoa, but that it still is the best criterion.

Abnormal Spermatozoa

The presence of large numbers of abnormal spermatozoa in the ejaculate of a male may indicate spermatic derangement or abnormality of the reproductive tract, with consequent reduction in fertility. When the animal is examined for such abnormalities allowance must be made for such factors as season of the year, previous sexual activity, and method of collecting semen. McKenzie and Berliner (*43*) show that for rams the number of abnormal spermatozoa is much higher out of the regular breeding season, some rams having as many as 700 to 900 abnormal spermatozoa per 1,000 during June (fig. 2). Lewis (*41*) made a seasonal study on fertility of Holstein and Guernsey bulls. The nonreturns (inseminated cows supposedly becoming pregnant since they did not return to heat) when Holstein bulls were used were 6.3 percent higher than when Guernsey bulls were used, both breeds being low in winter and summer. Mercier and Salisbury (*48*) found that fertility of young bulls was lower in summer and that of old bulls was lower in winter.

The breeding activity of males may also influence the abnormal spermatozoa content of semen. Spermatozoa of males that do not copulate frequently (within 14 days or longer) collect in the efferent ducts, especially the ampulla of the vas deferens, where they eventually undergo degeneration. At least two ejaculates should be collected from such males. An interval of 10 minutes or longer should be allowed between ejaculates, these being kept separate for examination.

The manner of collection also influences the abnormality count as well as the total volume of semen collected. If collection is made directly from the vagina, semen from a previous service might be mixed with the fresh ejaculate. Since the life of spermatozoa is

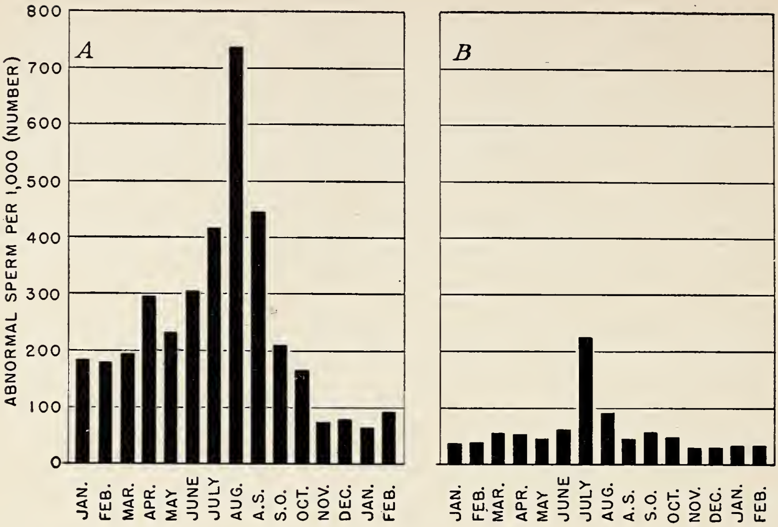

FIGURE 2.—Influence of season on production of abnormal spermatozoa in sheep, January 1935 to February 1936. The counts in each period (January to February, inclusive) were made every 28 days, resulting in considerable overlapping during August to October: *A*, Shropshires; *B*, Hampshires. Adapted from Missouri Agricultural Experiment Station Research Bulletin 265.)

relatively short in the vagina, if collection is made from a female that has been served less than about 30 hours earlier, sufficient degenerated spermatozoa from the earlier service may be collected to increase greatly the abnormal count of the male under test. Fresh semen collected in the artificial vagina is the most reliable source for examination. Starke (*77*) states that a coiled tail ranging from envelopment of the head to curling of the end of the tail was the most frequent type of abnormality and the most changeable in ram semen.

In his systematic examination of semen from most of the Danish bulls used in artificial insemination, Blom (*12*) has described the abnormalities found. Blom and Christensen (*13*) obtained the reproductive tracts of more than 2,000 slaughtered bulls for a further study of the origin of the abnormalities and pathology of the sexual organs. Blom's description (*12*) includes normal spermatozoa and free cytoplasmic droplets (fig. 3, *a* and *b*). He classified the abnormal conditions as primary spermatozoa abnormalities (*c*), secondary spermatozoa abnormalities, (*d* to *h*), and cells other than spermatozoa occurring in semen (*i* to *n*).

The primary abnormalities were due to disorders in the spermatic epithelium. In 100 normal fertile bulls Blom found from 4.65 to 10.4 percent primary abnormalities. In bulls in which more than 15 percent were found, the condition was accompanied by impaired fertility and in many instances by either testis degeneration or testis hypoplasia. Blom and others have observed a strongly refractive acrosomal formation at the anterior margin of the sperm head (an acrosome). Blom classes this as a primary abnormality. It has been found in sterile bulls. Hancock (*31*) found evidence that this condition of sterility was hereditary in a family of Fresian bulls.

FIGURE 3.—Outline drawing from Blom (*12*) showing normal and abnormal contents of bull semen: *a*, Normal spermatozoa; *b*, free cytoplasmic droplets; *c*, different types of primary sperm abnormalities; *d–h*, secondary sperm abnormalities—*d*, loose normal heads; *e*, spermatozoa with proximal droplet; *f*, spermatozoa with distal droplet; *g*, bent tails; *h*, detachment of the galea capitis; *i–n*, cells other than spermatozoa occurring in semen—*i*, medusa formations; *k*, desquamated epithelial cells from the prepuce or canalis urogenitalis; *l*, primordial spermiogenic cells (from cases of severe testis degeneration); *m*, erythrocytes; *n*, pus cells. (Enlarged about 1,000 times.)

In the secondary spermatozoa abnormalities, Blom described five forms appearing as a result of adverse conditions after the spermatozoa left the spermiogenic epithelium. These may appear during their stay in the epididymis, during ejaculation, or in subsequent manipulations. The loose normal heads (fig. 3, *d*) may be artifacts, but a tentative estimate of the number occurring normally may be made when examining fresh semen under a cover slip. A varying number may be found in practically all samples, but in many cases an increase in these forms

is an indication of testis degeneration. Immature spermatozoa (proximal droplet, fig. 3, *e*), according to Lagerlöf, must be regarded as pathological and should not be present in numbers greater than 2 to 3 percent. The distal droplet, *f*, apparently should have disappeared by the time of ejaculation, but 20 to 25 percent of such forms have been observed in semen from bulls of good health and fertility. Bent tails on spermatozoa, *g*, was the most common abnormality found. A constant content of 30 to 50 percent was generally accompanied by impaired fertility. One cause of this condition is believed to be cold shock in the process of collection. The collection tube should be kept between room and body temperature. Detachment of galea capitis, *h*, has been demonstrated in slow-breeding bulls and after breeding abstinence of 6 months or longer.

Cells other than spermatozoa were rather limited in normal bull semen. The medusa formations (fig. 3, *i*) are detached fragments of the ciliated cells lining the epididymis and are expelled with the semen at the rate of about 1 per 10,000 spermatozoa in normal bulls. In stallions the occurrence is more frequent. Centrifuged sediment from semen samples of extremely low concentration may contain 25 per 100 spermatozoa, which indicates severe pathological conditions in the testis epididymis. Epithelial cells *k*, may occur in normal semen. The primordial spermiogenic cells, *l*, may be common in severe testis degeneration. Blood cells, *m*, indicate an injury. Pus cells, *n*, indicate an infection in the genitals, most frequently in the seminal vesicles or the ampulla.

Bretschneider (*18*) gives a more detailed study, through the use of the electron microscope, of the abnormalities described by Blom. With this information at hand, research should be directed toward treatment, feeding, and handling of bulls for the purpose of restoring fertility in affected animals and of maintaining fertility in all individuals.

Preparation and Staining of Spermatozoa

For ordinary microscopic examination for abnormalities, a small drop of well-mixed semen is placed on a microscope slide in a film sufficiently thin that the spermatozoa can be examined individually with oil emersion. The semen may be spread by drawing the edge of a cover slip in the same manner that blood smears are made. Another method consists in gently dropping another slide onto the semen and then separating the two slides. As reported by Blom in correspondence with the author, a small drop of semen can be most satisfactorily spread on a slide without excessive mechanical artifacts by using a glass rod about 3 mm. in diameter. The rod is laid across the slide and the semen spread to the desired thickness by short forward movements. The slide is then labeled and set aside in a clean, dust-free place to dry, after which it is stained for examination.

Staining Technique

Staining may be carried out by any of the following methods:

1. Dried semen smears may be stained satisfactorily with 0.5-percent alcohol solution of gentian violet for 3 minutes.

2. The slide may be air-dried, put in a saturated solution of chlorazene for 5 to 10 minutes, rinsed in distilled water, fixed in 10-percent formalin for 3 to 5

minutes, rinsed in distilled water, stained with Ziehl's carbol-fuchsin for 2 minutes, washed gently in running tap water, and dried.

3. Lagerlöf (*38*) gives the following instructions for demonstrating protoplasmic drops in bull semen: Immediately after collecting the semen, place two drops on a slide and mix with it three drops of opal blue (Bresslau). Immediately before staining the semen, heat the opal blue until it steams, then filter it and cool to near room temperature. After the stain and the semen have been mixed, spread and allow to air-dry. Examination may be made with the slide wet or dry.

4. Blom (*11*) developed positive and negative (page 17) staining techniques for examination of semen abnormalities. For a positive stain, a suitable smear must be fixed and stained immediately. The preparation is fixed by being passed several times through a flame or through alcohol. It is then washed in flowing distilled water. For staining purposes, two solutions made up with distilled water are used. The one includes 1-percent sodium carbonate (Na_2CO_3 anhydrous) and the other, 1-percent methyl violet (stain quality 6B, Grüblers). One part of the first solution is mixed thoroughly with nine parts of the second. This stain is poured onto the still moist preparation and left for 4 to 5 minutes. It is then poured off, and the preparation is washed with flowing distilled water. Three or four vigorous jets from a wash bottle are suitable. It should be washed not more than 10 to 15 seconds in all, then blotted with filter paper, dried by passing the slide two or three times through the flame, and finally mounted with neutral Canada balsam for preservation. Although the methyl violet solution will keep for a year or more, after being mixed with the sodium carbonate solution it must be used immediately.

Rao and Hart (*64*) give a method of counting the live and the dead spermatozoa with the hemocytometer slide. They used this technique to check the accuracy of the opal blue-eosin staining mixture described by Lasley and coworkers (*40*), which is a negative stain for determining the numbers of live and dead spermatozoa. It was found that the variations in the two methods were consistent. The opal blue-eosin method gave a higher percentage of live spermatozoa because the nonmotile, living spermatozoa did not take the stain.

A staining method using fast green to replace opal blue (unobtainable during World War II) has been developed by Mayer, Squiers, and Bogart (*47*). They found this stain superior to the former opal blue mixture. According to Mayer in correspondence with the author, the staining mixture consisted of 0.8 gm. of eosin B [6] and 2.0 gm. of fast green (FCF) in 100 cc. of isotonic sodium citrate buffer or sodium phosphate buffer. The new mixture worked well on human, stallion, bull, and ram semen. Some difficulty was experienced with boar semen because of the gelatinous protein material present. The author has used this stain extensively, under field conditions, for examination of semen from bulls. When it is impossible to collect specimens in the artificial vagina, semen samples may be taken from the vagina of the cow, stained immediately, and examined later. About three drops of stain to one drop of vaginal mixture is used. The mucus-semen mixture may be cut to size by using the edge of an extra slide.

Shaffer and Almquist (*75*) have substituted aniline blue for opal blue in a staining mixture. From different combinations tried, a final staining mixture consisting of 1 percent of eosin B and 4 percent of aniline blue dissolved in M/8 phosphate buffer having a pH of 7.2 was selected as a satisfactory differential stain for bull spermatozoa.

For a negative stain to demonstrate secondary sperm abnormalities of protoplasmic drops, strongly refractive acrosomal formation, and loss of galea capitis, Blom, as communicated to the author, uses the

[6] Obtained as Eosin B Bluish from The Coleman and Bell Co., Norwood, Ohio.

following technique which also differentiates between live and dead spermatozoa. A small drop of semen, an equal-sized drop of 5-percent eosin in distilled water, and about three times as much 10-percent nigrosine in distilled water are placed in different positions on a microscope slide. The eosin and semen are first mixed with a glass rod, and immediately afterward the nigrosine is stirred thoroughly into the mixture. The mixture is then spread over the slide to the desired thickness with the glass rod. The smear is dried quickly and mounted with neutral Canada balsam.

Blom has also used India ink as a background stain. It can be substituted for nigrosine in the above procedure. The ink should be free from bacteria and consist of a finely ground homogenous mixture.

Number of Spermatozoa

The number of spermatozoa per given unit of semen (table 1) may be most accurately determined by means of a hemocytometer. For this examination the semen is diluted 100 or more times with 0.9-percent saline solution by means of a special pipette. After dilution and thorough mixing, a drop of the solution is placed on the counting chamber, and the spermatozoa are counted for a definite number of squares on the counting chamber. When this number is obtained, the number per cubic millimeter of semen may be calculated.

The apparatus used for this examination must be clean, dry, and free from grease, and the semen should be well-mixed before a sample is taken. After the semen and saline solution are drawn up in the pipette, they should be well shaken and the first three or four drops of the mixture discarded, since these drops are not likely to represent a well-mixed sample of semen and saline. The drop placed on the counting chamber should not be too large, and the layer of fluid should be of an even thickness under the cover slip before the counts are made. Only spermatozoa whose heads lie within the squares should be counted. Of those heads that lie upon lines of demarcation between squares, only those on two sides of the squares are counted; heads lying on the lines demarking the other two sides of the square are not counted.

An adequate technique for counting bull and ram spermatozoa is carried out as follows, all operations to be conducted at room temperature:

PROCEDURE

1. Stir sample well.
2. Take 0.1 cc. with 0.2-cc. pipette graduated in 0.1-cc. units.
3. Add 9.9 cc. of 0.9-percent sodium chloride from a burette (thus obtaining one one-hundreth dilution of the semen).
4. Take 0.1 cc. of the diluted semen of step 3.[7]
5. Add 9.9 cc. of 0.9-percent sodium chloride (thus obtaining one ten-thousandth dilution).
6. Take up in pipette with rubber bulb, after agitating several times by pressing and releasing bulb; waste a few drops; then with equal pressure put a drop on the counting slide.
7. Wait 10 minutes for spermatazoa to settle.
8. Make three counting slides.
9. Count 16 to 64 squares in each slide, the number of squares counted depending on whether or not the sample is concentrated.

[7] Steps 4 and 5 are omitted in preparing horse and boar semen for counting, a dilution of 1 : 100 being sufficient.

CALCULATION

0.2 mm. (marked on slide) times $\frac{1}{16}$ sq. mm.=$\frac{1}{80}$ cu. mm.

Number of spermatozoa per square=$\frac{a}{b}$(a=number of spermatozoa; b=number of squares counted)

Number of spermatozoa per cubic millimeter=$\frac{a}{\frac{1}{b \times 80}} = \frac{a \times 80}{b}$

Or, number of spermatozoa per cubic centimeter (or density)=$\frac{(a \times 80)}{b} \times 1{,}000$

Multiply by 10,000 to get the number of spermatozoa per cubic centimeter, because dilution is 1 to 10,000.

For example,	21 spermatozoa in	16 squares
	27 spermatozoa in	16 squares
	24 spermatozoa in	16 squares
	72	48

Density=$\frac{72 \times 80}{48} \times 10{,}000$=1,200,000 spermatozoa per cubic millimeter, or 1,200,000,000 per cubic centimeter.

Hammond and coworkers (*30*) suggest a more rapid, but perhaps less accurate, estimate of density by comparing the opacity of a diluted sample of semen with permanent standard opacity tubes, which can be prepared in any laboratory. A dilution of one part semen in nine parts saline will bring most samples within the opacity range of most standard tubes. Their method of comparison was made by gauging the facility with which small print could be read through the tubes and not by color comparison.

OTHER EXAMINATIONS OF SEMEN

Methylene-Blue Reduction Test

The methylene-blue reduction test is an index of both duration of motility and concentration of spermatozoa. This test is made by mixing 0.2 cc. of semen in 0.8 cc. of egg-yolk citrate diluter (see Use of Diluters) and adding to this 0.1 cc. of methylene-blue solution by dissolving 50 mg. of methylene blue in 100 cc. of sodium citrate diluter. This mixture is placed in a water bath at 37.8° to 46° C. (100° to 115° F.). The test is based on the rate at which the semen reduces or bleaches out the dilute solution of methylene blue (green in egg-yolk diluter) to the original yolk color. Good semen reduces the color in 3.5 to 6 minutes. Poor semen requires 9 minutes or longer.

VanDemark and coworkers (*84*) found highly significant correlations between the methylene-blue reduction time and volume of the ejaculate, spermatozoa count, initial spermatozoa motility, initial pH, and the initial lactic-acid level of fresh semen. Also, in diluted semen equally significant associations were shown between the methylene-blue reduction time and the lactic-acid gain and survival of spermatozoa after an hour's incubation at 46.5° C. as well as after 10 days' storage at 5° C.

Hydrogen-ion Concentration

The hydrogen-ion concentration can best be determined by an electric potentiometer with calomel cell and quinhydrone. However, if it is not available the pH may be closely estimated by adding 8 drops of 0.2-percent bromothymol blue in 90-percent alcohol to 1 cc. of freshly collected undiluted semen and comparing the resultant color with a standardized color chart. A yellow color indicates a pH of 6.0 or lower; yellowish green, about 6.4; medium green, about 6.8; bluish green, about 7.2; and blue, 7.6 or higher.

Raps and Cannon (*66*) made a study of the pH of 371 semen samples from 66 bulls in 5 studs for a period of 11 months and found that it was influenced by both management of the stud and season of the year. Reid and coworkers (*68*) found that the change in pH resulting from incubation of semen at 37° C. for 1 hour is probably the best simple quick test of semen quality available at the present time. A good review of literature is given in the articles cited.

A pH of 7.0 or above may indicate excessive use of the animal for breeding, the presence of urine in the semen, or incomplete ejaculation, which consists largely of accessory gland secretions (prostate and bulbo-urethral). Blom and Christensen (*13*) state that when these possibilities can be ruled out, a change of pH in the alkaline direction to 7.4 or higher suggests an inflammation, likely in the vesicular glands, or low fertility or sterility of the semen. In acute bilateral vesiculitis the alkaline reaction will be pronounced. The hydrogen-ion content of normal semen is indicated for each of several species in table 1.

Bromothymol Blue-Catalase Test

This test as described by Blom and Christensen (*13*) is used to determine the cleanliness of bull semen. It is based on the fact that normal, cleanly collected semen contains small amounts of the hydrogen peroxide splitting enzyme, or catalase. A rise in the catalase content of semen may be due to the presence of pus, blood, bacteria in large amounts, or to the pollution in the collecting process. The test is conducted as follows:

1. 1 cc. of the freshly collected undiluted semen is transferred to a catalase tube (fig. 4). (The pH of this semen may first be obtained by adding bromothymol blue.)
2. Next add 3-percent hydrogen peroxide solution to the ring or mark above the last graduation (near stopper).
3. The special stopper, provided with a capillary tube, is inserted to the first or double ring. This leaves a space between stopper and liquid equal to the space below graduation in the bottom of the tube.
4. The catalase tube is shaken vigorously three or four times, the opening of the capillary tube being closed by a finger. This tube is then placed in a rack, bottom up, and the time recorded.
5. At intervals of 5, 10, and 15 minutes the tube is shaken vigorously as above.
6. After 20 minutes the reading is taken at the junction between foam and fluid. If the lowest graduation (2,000 mark) is passed within 20 minutes the time is recorded when this happens. If 0.5 or 0.25 cc. of semen is used the result must be multiplied by 2 or 4, respectively.

The catalase number of normal, cleanly collected bull semen should be less than 300. Catalase numbers between 300 and 400 are suspect. Catalase numbers higher than 400 are highly indicative of inflamma-

tion in the genitals of the bull, the presence in the semen of blood from a scratch on the penis or other injury, of dirt, or of feces, or a secondary bacterial growth after collection.

Bacterial Contamination

Semen samples collected in the artificial vagina are subject to contamination with bacteria. Many varieties may be found, as shown by Gunsalus and coworkers (*27*), Morgan and associates (*53*), and Edmondson and associates (*24*). They may come from the reproductive organs of the male, the artificial vagina used for collection, or the surrounding air. However, the bacterial count in semen from healthy bulls can be kept very low by sanitary measures, such as clean quarters for collection, regular grooming, limited use of a lubricant at the opening of the artificial vagina, and sterile apparatus for collecting. As an added precaution, antibiotics may be used to help control most varieties of bacteria found in semen (p. 25). If harmful organisms are suspected, a complete bacteriological analysis should be made. If they are found, a clinical examination of the bull should be conducted to locate the source of infection.

Serological Test

Bendixen and Blom (*7*) state that the agglutination test may be used for detecting *Brucella* agglutinins in the semen. This procedure is similar to the one used on blood serum, except that the spermatozoa are killed with a 1-percent aqueous solution of sodium azide before centrifugation. Some bulls positive to the blood test may also react to the semen test. A positive reaction in the semen may indicate localized infection in the genital tract.

HANDLING THE SEMEN

If inseminations are not to be made immediately after collection of semen, precautions must be taken to keep the spermatozoa in a high state of viability if the largest percentage of conceptions is to ensue. Temperature shocks and contact with water and harmful chemicals should be avoided, and the sample should be protected as much as possible from contact with air.

Undiluted Semen

In early work the manner of handling undiluted semen depended largely on the interval between collection and insemination. If insemination was to be made within 2 hours after collection, precautions were relatively simple. It was sufficient merely to place the semen in a small stoppered vial that had been thoroughly cleansed and dried.

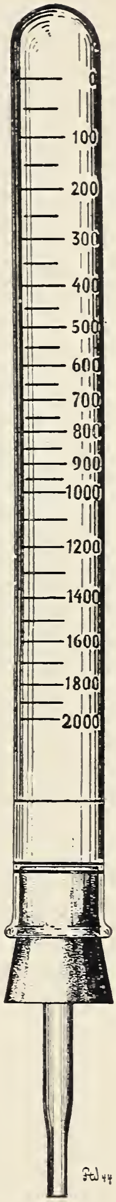

Figure 4.—Catalase tube fitted with rubber stopper and capillary tube. From Blom and Christensen (*13*).

The vial was then left at room temperature in a closed container or in a dark place and under no conditions exposed to direct sunlight. If the semen was to be kept for longer periods, the following precautions were taken.

Immediately after collection the semen was placed in a clean vial and covered with a layer of high-grade neutral paraffin oil up to the cork, thus eliminating any air space in the vial. The vial was then wrapped in two thicknesses of paper and set in the refrigerator, or the paper-wrapped vial was covered with two rubber thumbstalls and dropped in a vacuum flask containing water at 3° to 8° C. This provided for a gradual cooling of the sample and maintenance temperature at 3° to 8° C. The equipment needed is shown in figure 5. The

FIGURE 5.—Equipment for transporting semen: *a*, Wide-mouthed, quart vacuum bottle with cork at extreme left; *b*, small glass vials for holding the semen; *c*, paper and two thumbstalls for wrapping the vial (vial at left is shown labeled and wrapped); *d*, top for the vacuum bottle.

use of paraffin oil may be avoided by using paraffin wax plugs pressed into the vials down to the semen. Two or three drops of melted paraffin is poured over the wax plug to seal the vial.

The above-described technique has been successfully used with bull, ram, and goat semen, but different storage methods were used for the more watery types of semen such as those of the stallion and boar. Walton and Prawochenski (*86*) have satisfactorily stored stallion semen 24 hours by first separating out the glairy, viscous portion of the ejaculate, centrifuging the rest, and storing it at 0° to 3° C. At the Missouri station boar semen has been successfully stored for 56 hours by removing the gelatinous lumps and adding to the remainder an equal volume of the swine diluter described by Milovanov (*50*, *51*) and given in tables 2 and 3. Storage was at 10° to 12° C.

Use of Diluters

The primary purpose of diluters is to increase the volume of the ejaculate of a male so that it may be used to inseminate a larger number of females. A good diluter must not be toxic to spermatozoa; the osmotic relations must be similar to those in the undiluted semen; the hydrogen-ion concentration of the solution must be favorable for

continued viability of the spermatozoa; it should contain a buffering solution to protect against marked changes in the hydrogen-ion concentration; it should increase the length of time that semen can be stored without loss in fertility, prevent injury from cold shock, and be inexpensive and easy to prepare. Milovanov (*50*) has described diluting solutions which apparently meet most of these requirements satisfactorily. Formulas for diluters developed by Milovanov (*51*) for those species in which artificial insemination has been used most extensively are listed in tables 2 and 3.

TABLE 2.—*Formulas for tartrate diluters for semen of farm animals*

Animal	Glass-distilled water	Anhydrous glucose ($C_6H_{12}O_6$)	Sodium potassium tartrate ($KNaC_4H_4O_6$)	Peptone (salt-free)
	Grams	*Grams*	*Grams*	*Grams*
Stallion	1, 000	57. 0	6. 7	2. 0
Bull	1, 000	12. 0	25. 6	5. 0
Boar	1, 000	46. 1	5. 6	3. 5
Buck (rabbit)	1, 000	39. 0	7. 0	2. 0

TABLE 3.—*Formulas for sulfate and phosphate diluters for semen of farm animals*

Animal	Glass-distilled water	Anhydrous sodium sulfate (Na_2SO_4)	Anhydrous glucose ($C_6H_{12}O_6$)	Peptone (salt-free)	Disodium phosphate Na_2HPO_4 ($12H_2O$)	Monopotassium phosphate (KH_2PO_4	Calcium lactate ($CaC_6H_{10}O_63H_2O$)
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Stallion	1, 000	3. 4	57. 6	2. 0			
Bull	1, 000	13. 6	12. 0	5. 0			
Ram	1, 000		50. 4		6. 78	0. 15	1. 91
Boar	1, 000	2. 8	46. 1	3. 5			
Buck (rabbit)	1, 000	3. 6	39. 0	2. 0			

The phosphoric acid-calcium sediment which is precipitated in the sheep-semen diluter is filtered off. The optimum dilutions for these solutions, as reported by Milovanov (*50*), are, respectively, in parts of semen to diluter: Stallion, 1 to 7; bull, 1 to 15; ram, 1 to 31; boar, according to Rodin and Lipatov (*69*), 1 to 4; and buck rabbits, 1 to 15.

A diluter containing added egg yolk, developed by Lardy and Phillips (*39*), for use in handling bull semen, has served as a nucleus around which other formulas have been developed. Their original egg yolk-phosphate formula is as follows:

To 100 cc. of boiling distilled water add—
  0.2 gm. KH_2PO_4 (chemically pure).
  2.0 gm. $Na_2HPO_4.12H_2O$ (chemically pure).

After this mixture has cooled to room temperature, add an equal volume of fresh egg yolk which has been carefully separated from the whites.

The development of this formula was closely followed by the development of the egg yolk-citrate diluter by Salisbury, Fuller, and Willett (*73*). This diluter had a quality superior to that of the phosphate formula. It dispersed the fat globules of the egg yolk so that the individual sperm could be more readily seen microscopically. Knodt and Salisbury (*37*) recommended a concentration of 3.6 gm. $Na_3C_6H_5O_7.2H_2O$ per 100 ml. of water distilled in glass to be used with an equal amount of egg yolk. With this formula, sperm motility was preserved equal to that in semen stored in egg kolk-phosphate formula for 5 days but was slightly greater in semen stored 6 days or longer.

Swanson (*80*) found that 3-percent sodium citrate dihydrate was superior to other concentrations tried and that by reducing the egg yolk concentration to 20 percent it gave as good survival and motility as the commonly used 50 percent. When the egg yolk was reduced to 10 percent, the cold-shock protective factor was still fully effective, but motility was impaired slightly.

A more recent diluter has been formulated by Phillips and Spitzer (*60*), as follows:

	Percent
Glucose	0.6
Galactose	.2
KH_2PO_4	.2
$Na_2HPO_4 12H_2O$	2.0
Lipositol, or purified lecithin	1–2.0
Gum acacia	3.0
Distilled H_2O to volume	
Sulfathalidine, sulfasuxidine, or streptomycin	.03

This lipide-glucose-buffer-gum solution can be prepared by weighing out the glucose, buffer salts, lipositol, and gum and then adding the appropriate amount of distilled water. The mixture is boiled gently and then the antibiotic is added. The keeping quality of semen in this diluter was comparable to that for egg yolk buffer. It has the desirable feature of making the spermatozoa plainly visible.

A delay in either the diluting or the cooling process has been found to lower desirable characteristics of semen. Hurst and LaMaster (*34*) found little difference in percentage of pregnancies between different diluters used for semen storage, but did find that the age of the semen used made a difference. After the results of three diluters tried were pooled, semen up to 35 hours of age gave 70 percent nonreturns (65-day basis) and those more than 35 hours of age gave 46 percent nonreturns.

For horse and jack semen the Missouri Agricultural Experiment Station recommends the use of the egg yolk-phosphate formula of Lardy and Phillips (*39*) (p. 23) except that to the 100 cc. of boiling distilled water 10 gm. of dextrose or glucose (chemically pure) is added. One part of horse or jack semen is diluted with one part of this glucose and egg yolk buffer. This dilution is made immediately after collection and before refrigeration.

Berliner (*9*) found that the Russian diluter consisting of 5.76 gm. of anhydrous glucose and 0.67 gm. of potassium-sodium tartrate (TGL) was much improved by the addition of the yolks of two eggs

per 100 cc. for stallion semen, but the spermatozoa did not withstand transportation. The later addition of 1.8 gm. of gelatin (½-ounce gelatin capsule) to 100 cc. of diluter improved the mixture in this respect.

For ram semen, Terril, as reported by Perry and coauthors (*58*), states that the egg yolk formula developed by Phillips and Lardy is probably the most satisfactory diluter available. The semen should be diluted, cooled (avoiding temperature shock), and stored at 2° to 5° C.

Bonnier and Trulsson (*16*) found that cock semen can be diluted without lowering fertility provided it is used within an hour after collection. In these studies 1 cc. of semen was diluted with nine parts of modified ringer solution. The modified ringer solution was made as follows: In 10,000 cc. of distilled water were dissolved NaCl, 68 gm.; KCl, 17.33 gm.; CaCl, 6.43 gm.; $MgSO_4$, 2.5 gm.; and $NaHCO_3$, 24.50 gm.

Stimulants for Increasing Activity of Spermatozoa and Conception Rate

Certain substances stimulate spermatozoa at certain concentrations. Strychnine hydrochloride was found to stimulate guinea-pig spermatozoa strongly at concentrations of 0.16 and 0.64 percent and less strongly at 0.256 percent. Spermatozoa so stimulated were not damaged and gave rise to normal offspring. This drug has a decidedly stimulating effect on ram and bull semen stored for 3 days. Although further experimental work is needed before conclusions can be drawn, these and other solutions may bring about fertility of valuable males whose sterility is due to feeble spermatozoa, and offer promise of restoring vigor to spermatozoa that have lost their vitality in storage.

Schultze and Davis (*74*) increased the percentage of nonreturns to service after 5 months by the addition of thyroxine (10 micrograms percent of d, l'thyroxine) to diluted semen. The increase in fertility appears to be maintained for a 4-day storage period.

Effects of Antibiotics on Bacterial Content of Bull Semen

Knodt and Salisbury (*37*) have shown that 300 mg. of sulfanilamide added to 100 cc. of yolk citrate diluent improved the survival of spermatozoa. They reported that the sulfanilamide effectively controlled most of the bacteria normally found in semen. They later found that the beneficial effects of sulfanilamide on fertility were largely due to metabolism rather than to bacterial control alone. Foote and Salisbury (*25*) used 12 sulfonamides in bull semen diluted with citrate buffer. Each drug was added at an optimum level for spermatozoa survival. Nine increased survival; two—sodium sulfamethazine and carboxysulfathiazole—were significantly superior to sulfanilamide in maintaining motility of spermatozoa but were inferior in controlling bacterial content. *Pseudomonas aeruginosa* was not inhibited at 37.5°, 20°, or 5° C.

Almquist and coworkers (*2*) found that a combination of penicillin and streptomycin, in levels of 100 to 1,000 units each per cubic centimeter of diluted semen, did not affect significantly the survival of bull

spermatozoa during a 20-day storage period. After a storage period of 8 days, this combination controlled bacteria commonly found in semen (pages 21 and 66) better than either one alone, as shown by earlier trials.

Bacteriological experiments show that optimum bactericidal quantities of streptomycin or penicillin added alone in the test tube may not kill certain types of bacteria, but their combination may kill the organisms. The time required for killing organisms may be 2 days or longer. By this time most semen has been used. Although their addition to semen from bulls of low fertility has given a slight increase in conception rate, this is possibly due to the control of these drugs over most of the bacteria in samples with high bacterial counts.

Addition of Dyes for Semen Identification

In artificial insemination of dairy cattle it is impossible to distinguish the semen of one breed from that of another by any characteristic of the semen. Thus, unless it can be readily identified by some outside means, semen of one breed might inadvertently be used to inseminate cows of a different breed. Phillips (*59*) found that Nile green sulfate, neutral red, and Sudan III, when added in sufficient amounts to color the diluted semen distinctly, had no appreciably detrimental effects on spermatozoan motility during several days of storage. Almquist (*1*) found that the following certified food, drug, and cosmetic liquid colors,[8] in not less than the designated pure dye percentages, gave adequate color differentiation when added to diluted semen: Strawberry shade red, 5.2 percent; emerald shade green, 2.7 percent; purple shade grape, 4.1 percent; and brown, 3.7 percent. One drop of dye to 10 ml. of diluted semen had no detrimental effect on motility of spermatozoa. Lower concentrations of dye had no significant effect on fertility.

The National Association of Artificial Breeders has adopted the following colors to be added to semen to designate the various breeds: Green for Holstein, purple for Ayrshire, brown for Brown Swiss, red for Jersey, and the uncolored egg yolk for Guernsey.

SHIPMENT OF SEMEN

It has become routine practice for large artificial-insemination associations to ship semen, for overnight delivery, by parcel post and bus to their technicians. As stated earlier, the semen of superior bulls is regularly shipped to any location within the country supplied by regular air schedules. When the semen is properly diluted, cooled, and packed, shipments to foreign countries have also been successful. Good-quality semen from fertile males, especially bulls and rams, may be used for 2 to 3 days with almost as good results as the same semen used on the day of collection. With the present methods of handling semen its fertility rate decreases in geometric progression as storage time increases. Only a few cases have been cited in which pregnancies have resulted from semen stored 6 days or longer.

[8] Obtained from Warner-Jenkinson Mfg. Co., 2526 Baldwin Street, St. Louis 6, Mo.

For large-scale overnight shipment of bull semen, the previously labeled vials of semen are often wrapped in several thicknesses of wrapping paper and placed beside a pound can of solid ice, prepared by placing sealed cans of water in the freezing compartment of a refrigerator or in a deep-freeze unit. Semen and ice are inserted into an insulated paper bag made for preserving frozen products for short periods. The bag is placed in an insulated or heavy cardboard box made to hold quart packages of ice cream.

The commercially available small-size cork-insulated shipping package for ice cream has been used for long-distance shipping. A gallon can is placed inside this container, and in this can is placed a half-pint vacuum bottle. The labeled vials containing the semen are inserted into rubber fingerstalls and placed in the half-pint vacuum bottle, which is then filled with water at a temperature of 4.5° C. (40° F.). A small amount of excelsior in the water will prevent the vials from bobbing. Wire is secured to the bottle to hold it upright. It is then placed in the can and cracked ice placed around it. Added refrigeration can be obtained by placing a flat can of ice or frozen brine above the gallon can. The entire container should be precooled in a freezing unit. The water surrounding the semen vials prevents any sudden change in temperature. This container has maintained a satisfactory temperature below 10° C. (50° F.) in hot summer weather.

Perry and associates (*58*) described an efficient container developed by The American Scientific Breeding Institute, Madison, Wis. It is a pint-size, stainless-steel vacuum bottle, the type with a deep narrow neck. To the neck is welded a tube, also of stainless steel, which extends down into the liquid-carrying part of the bottle that contains a chemical mixture, the principal ingredient of which is ethylene diamine. Within this tube is space for the insertion of a glass test tube of 25- to 30-cc. capacity. Before use, the refrigerant is frozen by placing the container in a low-freezing unit. The optimum holding temperature is 4.5° to 7.5° C. (40° to 46° F.), usually for 72 to 100 hours.

The importation into the United States of semen from cattle, sheep, or other domestic ruminants or swine from countries where rinderpest or foot-and-mouth disease exists is not permitted. For those desiring to import semen from animals located in countries free of rinderpest and foot-and-mouth disease, information and permission should be requested from the Inspection and Quarantine Division, Bureau of Animal Industry, United States Department of Agriculture, Washington 25, D. C.

INSEMINATING THE FEMALE

The percentage of successful impregnations to be obtained by use of artificial insemination is largely determined by the care with which the procedures are carried out. In the first place, it is necessary that the semen contain large numbers of normal viable spermatozoa and that the females to be inseminated be normal, free from diseases of the reproductive system, and in good breeding condition. Other important factors are: Timing inseminations so that they coincide closely with ovulation, making more than one insemination during

long heat periods, and introducing an adequate quantity of semen into the proper region of the genital tract of the female.

To prevent the transfer of infection from one female to another by the instruments used to introduce the semen, all instruments should be thoroughly cleansed and disinfected after their use on each individual. As spermatozoa are quickly killed on coming in contact with disinfectants, other means of sterilizing equipment employed in handling semen must be used. Boiling, the use of live steam, and the hot-air oven are the most desirable methods of sterilizing the glass and metal instruments. Sufficient sterile equipment should be provided for each day's work.

Insemination should be made in clean, dust-free quarters to prevent exposure of the vaginal mucosa of the female to infectious agents that might be carried in dust particles. If inseminations are to be made in paddocks or barns, these may be temporarily freed of dust by sprinkling the floor shortly before inseminations are made. The equipment used in insemination should be cleansed and handled as described on page 7.

Relation to Estrus and Ovulation

The life of spermatozoa in the reproductive tract of the female varies greatly both within and between species. It seldom exceeds 40 hours and is usually much less. After ovulation it is generally considered that the time within which the ovum can become fertilized is very short, possibly 6 hours or less. For optimum chances of fertilization the spermatozoa should reach the ovary at the time of ovulation. The time required for them to reach the ovary is variable. In sheep, 20 minutes has been reported by some workers, whereas others give an average of 5 to 6 hours. A time interval of 6 hours should be allowed for most species.

The duration of heat in most species is short but it also varies considerably within species. Ovulation occurs late in estrus (table 4), and inseminations should be timed to coincide closely with this interval. Determination of the exact time of onset of estrus is often impossible, but the breeder can be reasonably sure of success if inseminations are made during the last half of estrus. The relationship between fertility and time of insemination (in relation to ovulation) in the rabbit is shown in figure 6. Ovulation occurs approximately 10 hours after coitus in this animal.

In females that have excessively long estrus periods, two or more inseminations may be advisable. It is good practice to serve a mare a second time if she is in heat 3 days after the first service; on the second and fifth days after the onset of heat; or, if the stallion is not being used to excess, on the fourth, sixth, and eighth days after the onset of heat. Zivotkov (*88*) obtained 80.5 percent of pregnancies in mares bred between 4 and 48 hours before ovulation, as determined by palpation of the maturing follicle. He reports that mares begin to go out of heat 6 to 12 hours after ovulation and that they will not accept the stallion after 24 to 48 hours. Breeding after ovulation results in few pregnancies. He recommends that the state of the ripening follicle be determined by means of palpation before insemination or service.

To do this successfully a knowledge of the reproductive organs and skill in manipulation are required.

The investigations of Andrews and McKenzie (*3*) emphasize the value of palpating mare ovaries. Some mares did not show a desire to mate, yet the ovarian cyclic changes continued and artificially inseminating these mares resulted in pregnancy. Other mares showed signs of estrus for 1 or more days, then went out for a day or so and came in estrus again. Ovulation occurred during the second phase of the "split" estrus. Still other mares showed estrus but failed to ovulate until they had gone out of heat. Artificial insemination subsequent to the heat period resulted in impregnation.

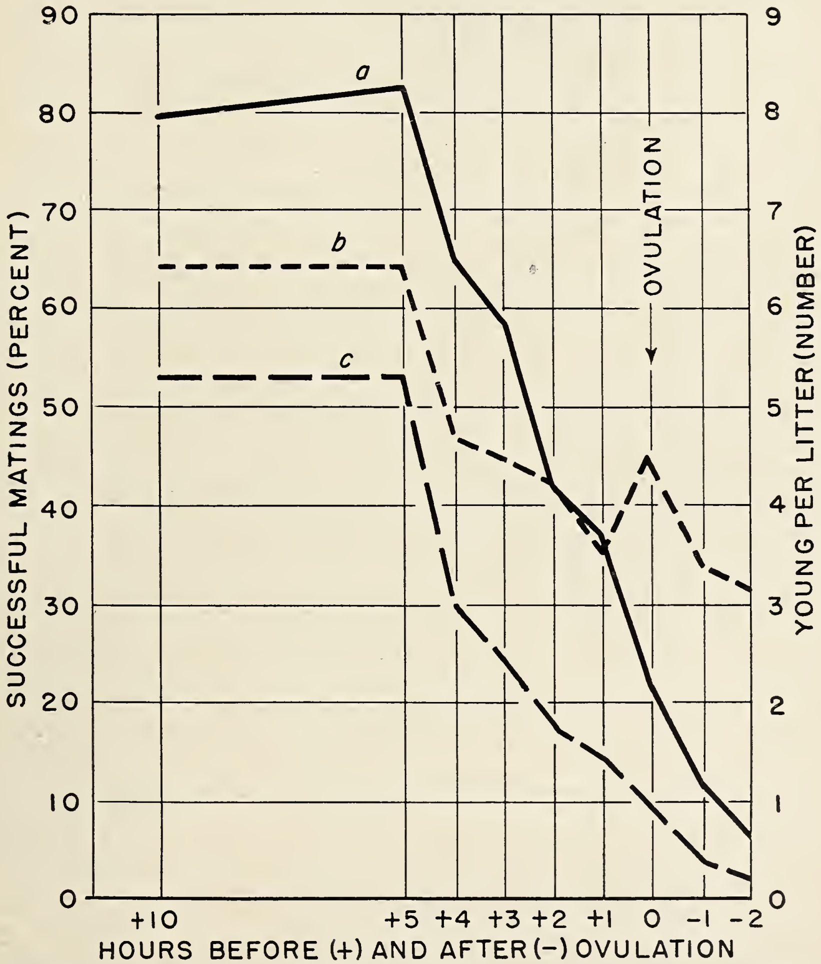

FIGURE 6.—Success of matings in relation to time of ovulation, for the rabbit. *a*, Percentage of matings that were successful; *b*, average litter size; *c*, number of young per mating. (In the rabbit, ovulation occurs approximately 10 hours after coitus.) (Adapted from Hammond, courtesy of the Journal of Experimental Biology.)

TABLE 4.—*Duration and frequency of estrus and time of ovulation in farm animals in normal condition*

Animal	Duration of heat				Length of estrual cycle [1]				Approximate time of ovulation in relation to heat	Optimum time to breed in heat period	Remarks
	Approximate range		Most common duration		Range		Most common duration				
	Days	*Hours*	*Days*	*Hours*	*Days*	*Hours*	*Days*	*Hours*			
Mare	1–37		3–7		10–37		18–24		2 days before end of heat until 1 day after heat.	If feasible, once daily after first day of heat in light mares, after second day in draft; if bred only once, on the third day. When the mare is in heat 3 days after breeding, breed a second time. If the ovary is palpated, breed when there is a large, slightly relaxed ovarian follicle, 2–5 cm. in diameter.	Foal heat in mares usually lasts 5 days, but may range from 1–10 days. Mares usually come into heat 5–10 days after foaling.
Cow		12–18		16	19–23		20		20–40 hours after onset of heat.	Preferably twice, once shortly after onset of heat and again 12–20 hours after onset; if but once, 12–20 hours after onset.	
Ewe		20–42		30	14–19		16–17		About 1 hour before end of heat.	During the last half of heat, or, if feasible, at 12-hour intervals as long as in heat.	There is evidence that the duration of heat is longer in some breeds, as the Lincoln, and Corriedale, the mean duration being about 40 hours.

Doe (goat)		20–80		39	12–27		19		Usually on the second day of heat.	During last half of heat period.	
Sow, mature	2–4		3		19–23		20–22		Early on the second day of heat.	Late on first day or preferably on second day of heat.	Sows usually come into heat 3–4 days after weaning pigs; earlier if the litter is small.
Gilt		40–48	2								
Bitch	4–13		9		126–240		180		24–48 hours after onset of heat (first acceptance of coitus).	On eleventh to thirteenth day after beginning to bleed.	Much individual variability exists in estrual cycle. Each bitch usually remains quite constant to 1 particular period. Length of cycle is influenced to some extent by such factors as breed and age.
Doe (rabbit)									8–10 hours after coitus.	Breeding again 2 to 5 hours after first coitus increases litter size. Breeding later than 5 hours after ovulation reduces litter size.	No regular estrual cycle exists, but there is evidence that there are certain periods of greater receptivity. If nutritive conditions are favorable, and the does are not molting and are in proper breeding condition, they may be mated at any time if restrained.

[1] The length of the estrual cycle is the interval from the beginning of 1 heat period to the beginning of the next.

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According to Milovanov (*52*), some Russian sheep stations experienced a large increase in lambing percentages when the ewes were inseminated several times, and Neiman (*56*) reported similar results in Karakul sheep. Also, according to Neiman, two inseminations of cows at intervals of 12 to 24 hours during one heat period resulted in 92.5 percent of pregnancies, as compared with 60 to 65 percent with a single insemination. When spermatozoa are of inferior quality such repeated inseminations are apt to be most effective.

Trimberger and Davis (*82*) artificially inseminated 295 cows at various stages of estrus to determine the effect of time of service on conception, with the following results:

The breeding results, expressed as percentages of conception from one insemination in the females bred at various stages of estrus, were as follows: Start of estrus, 44.0; middle of estrus, 82.5; middle of estrus and rebred in 24 hours, 84.0; end of estrus, 75.0; 6 hours after estrus ended, 62.5; 12 hours after estrus, 32.0; 18 hours after estrus ended, 28.0; 24 hours after estrus ended, 12.0; 36 hours after estrus ended, 8.0; and 48 hours after estrus ended, none conceived.

A large percentage of cows and heifers fail to come in heat at the time desired to breed them. Furthermore, intervals between heat periods may be too long. The owner's records may show that such females have not been bred, but many times these records are not complete and the apparent delay is due to pregnancy. In treating such cases stilbestrol and other estrogenic hormones are frequently used. These hormones induce abortion in pregnant females. Thus, many abortions are induced because a preliminary examination for pregnancy was not made.

Place of Injection and Quantity of Semen

Certain factors concerning the site of deposition of semen within the female should be considered. In natural service from the male, the ejaculate is deposited in the anterior portion of the vagina on or near the os or cervix, and in some species it may be deposited within the cervix. In horses, the semen is sometimes ejaculated through the cervix, which is characteristically short, into the uterus.

At the time of heat, when the female should be bred, there is an increased secretion of mucus, which becomes thin and flows from the reproductive tract. Spermatozoa naturally swim against currents; thus it is possible that they cleanse themselves of bacteria when swimming against the flow of mucus through the cervix. All samples of semen collected in the artificial vagina or otherwise for insemination purposes contain bacteria (p. 21). Many types of these bacteria have been isolated from aborted fetuses. Therefore, deposition of semen in the entrance of or well within the cervix, as described for various species (pp. 33 to 63), would simulate natural service more closely than deposition within the uterus, as is practiced by many artificial-insemination cooperatives. More experimental work is needed to determine the optimum location for deposition of semen within the female tract.

The quantity of semen needed for successful fertilization depends on such factors as its quality, including the number of viable spermatozoa; the state of the reproductive tract of the female; the region into which the semen is introduced; and the stage of estrus. Neiman (*55*) reports that in cattle more than 2 cc. did not increase the

percentage of fertility, and that 0.5 to 2 cc. was most effective if the semen was introduced into the cervix. Semen diluted 1 to 400 times has given good results, but Salisbury and Bratton (*72*) suggest that for bulls of high fertility the minimum number of spermatozoa per insemination should be 5 to 10 millions. Raps (*65*) reports that bull semen may be diluted 1 to 45 to 1 to 60 times for use in 0.3 to 0.5 cc. per insemination.

In sheep, the effective quantity was found to be 0.1 to 0.2 cc., although inseminations may be obtained with 0.05 cc. Zivotkov (*88*) states that in horses the quantity of semen should vary according to the age and size of the mare. He found that in young and small mares 10 cc. of semen is sufficient, whereas in older and larger mares 20 to 25 cc. is best. His results were more favorable with such quantities than with 3 to 5 cc. Perry and coauthors (*58*) found that in small mares 10 to 20 cc. of semen was sufficient in contrast to 30 to 40 cc. in large mares.

In swine, much larger quantities are required for successful impregnations owing to the exceedingly long and convoluted horns of the uterus, which sometimes reach a total length of about 78 inches. McKenzie, Miller, and Bauguess (*44*) found that 50 cc. of boar semen freed of gelatinous particles was sufficient. In rabbits, Walton (*85*) reports that satisfactory results are obtained with 0.5 cc. of semen which has been diluted as much as 32 times. In chickens, insemination with 0.1 cc. of semen once a week should result in fertility of 80 to 95 percent of the eggs.

Horses and Asses

The stallion is handled in the same way as in normal service, preferably by a groom who is familiar with the procedure to be followed. The mare also should be handled by a groom, and there should be assurance that she is in heat. If the mare is inclined to be nervous or unruly, hobbles, and in extreme cases a twitch, should be used to restrain her and avoid injury to the operator and the stallion, but if she is quiet and fully in heat there is little likelihood of trouble. Always handle breeding stock gently. Avoid startling any animal. Place the mare in such a position that she can see the stallion or jack approaching. It is good practice to wash the penis of the stallion at regular intervals with soap and water to remove any dried secretions ("scales") and then rinse well with clear water. The tail of the mare should be bandaged and tied. If collection is to be made from the mare, the area around the tail region should be washed well with soap and warm water or a mild solution of a nonirritating disinfectant in warm water. A very satisfactory one consists of 20 cc. of a high-grade coal-tar creosote per gallon of warm water. The tail region should then be rinsed with clean water. The lips of the vulva should be spread and swabbed with cotton.

The apparatus and supplies needed vary with the method of collection. The following list gives the essential items:

Hobbles, to restrain the mare.

A cotton tail-bandage, 5 feet long and 2 inches wide.

Two buckets of approximately 10-quart capacity and preferably of enameled ware.

A speculum. This item is not absolutely necessary but is desirable for its use makes it possible for the operator to observe the interior of the vagina and the cervix.

Equipment for the collection of semen:

An artificial vagina (Missouri-U. S. D. A. model); or one 6- or 8-ounce bottle with a two-hole rubber stopper, fitted with glass tubes and rubber tubing (fig. 7); or a breeder's bag. If a large number of collections are to be made it is desirable to have all types of collecting equipment on hand.

Equipment for insemination:

Gelatin capsules (2 and 4 drams; ½ and 1 ounce, i. e., Nos. 11 and 10) which should be kept in a clean, tightly stoppered glass jar; or a 20-cc. glass syringe with glass plunger fitted with a 20-inch ebonite or glass nozzle which has well-rounded and smooth ends. Metal syringes should be avoided.

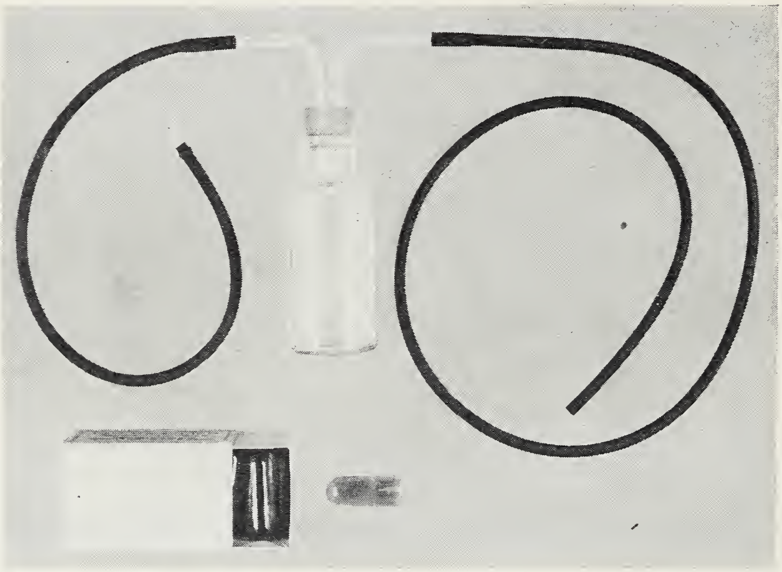

FIGURE 7.—Apparatus for aspirating semen from the mare and gelatin capsules (½ ounce) for insemination. A 6-ounce bottle with heavy walled gum-rubber tubing of 3/16-inch inner diameter is used. The gelatin capsules should be kept in a small, tight container such as a paper box or screw-top bottle. (Courtesy Missouri Agricultural Experiment Station.)

COLLECTION OF SEMEN

FROM THE VAGINA

This is one of the most satisfactory means for collecting semen from the horse, but precaution must be taken to see that the mare used for collection is free from disease. Nor should a mare in foal heat be used for collection since the danger from infection is too great at this time both for this mare and for those to be inseminated with semen so collected. A healthy mare that is in heat is hobbled and prepared as indicated above. After the male dismounts, the operator puts on rubber gloves, washes thoroughly, lubricates [9] the gloves and the vulvar

[9] A good lubricant that is also harmless to rubber and will not gravitate into the collecting tube is made by dissolving 100 gm. of gum tragacanth in 800 cc. of distilled water. To dissolve, add cold water and let stand in icebox overnight. Sterilize in the autoclave. If the lubricant is put in small receptacles, they can be discarded after they are opened and thereby contaminated.

lips of the mare, and with the long rubber tube of the semen collector (fig. 7) between the first and second fingers, carries the tube into the vagina and draws off the semen by aspiration, that is, by sucking on the short rubber tube. If the semen is within the cervix, the tube is guided into the cervix, and the semen is aspirated from there. If the weather is cold, protect the semen from sudden changes of temperature by keeping the semen bottle wrapped in a warm towel.

Another but less satisfactory means of collection following service is by use of the speculum and a syringe. After service a speculum may be carefully inserted into the vagina, opened, and then a nozzle which is attached to the syringe is carefully inserted into the vagina and the semen withdrawn into the syringe. The semen is deposited chiefly on the floor of the vagina just posterior to the cervix. After the semen is withdrawn it should be handled as described in the preceding paragraph.

FIGURE 8.—Breeder's bag. Size for draft horses is 15 by 3 inches (flat diameter of neck); for light horses, $13\frac{1}{2}$ by $2\frac{1}{4}$ inches. (Courtesy Missouri Agricultural Experiment Station.)

THE BREEDER'S BAG

If the stallion or jack will permit the use of the breeder's bag (fig. 8) it offers a very satisfactory and relatively simple means of collecting large volumes of semen free from female secretions. Place a breeder's bag of good quality and proper size on the penis while the stallion is getting the erection. Lubricate the outside bulb end of the bag and the external genitals of the mare. Permit service and after he has dismounted take the bag from the penis, pour the semen into a bottle, and cork it. Protect the material from direct sunlight and sudden changes in temperature.

BY THE ARTIFICIAL VAGINA

The advantages of collecting semen by means of the artificial vagina (figs. 9 and 10) over the previously described methods are that a greater quantity and better quality of semen are obtained and danger of infection is greatly minimized. The disadvantages are that the artificial vagina for the horse is rather expensive, especially if it is to be used only for occasional inseminations, and the temperature and pressure must be carefully regulated if the stallion is to be induced to use this device.

The Missouri-U. S. D. A. model of the artificial vagina for the horse consists simply of a rubber tube about 18 inches long and 7 inches in flat diameter with a rubber ring placed in the open end, and the other end narrowed down to stretch over a bottle. To develop the proper pressure, a second rubber tube of the same size is drawn over the first, the two tubes are then vulcanized together at each end, and an ordinary tire valve is placed in the outer tube so that air may be pumped between the two tubes. A leather casing surrounds the rubber tubing, giving it rigidity, and a handle grip is attached to the casing. An essential feature of this model is the 3-inch rubber band

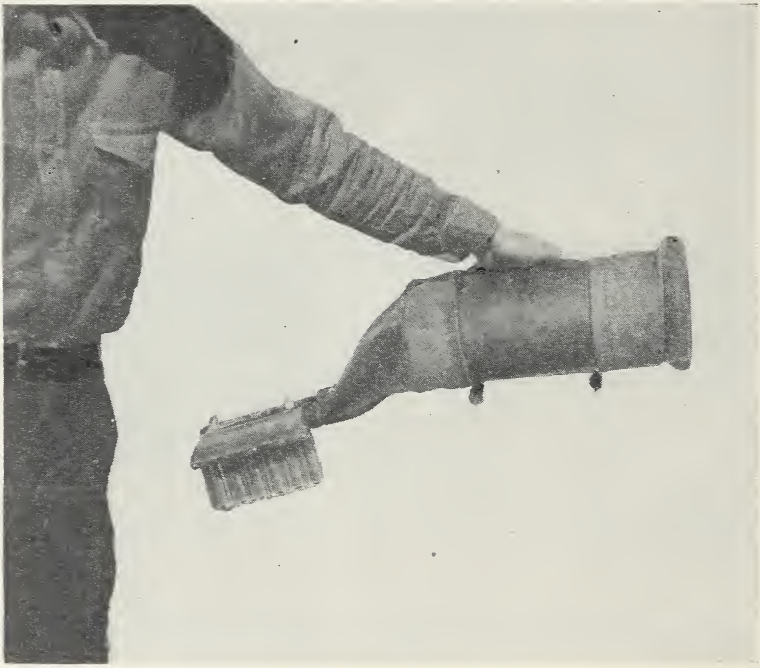

FIGURE 9.—Artificial vagina for the horse, Missouri-U. S. D. A. model. A device for fractionating the semen is attached to the lower (small) end of the apparatus.

placed around the inner tube near the open end. This simulates the sphincter muscles of the mare and aids materially in making collections from stallions. The apparatus is warmed by passing hot water through it just before use and flushing with either physiological saline solution (0.9 percent) or a good semen diluter. If the valve is large enough, pour a quart of hot water through it to warm the apparatus, and thus make flushing with saline unnecessary. Before attaching the collecting device, previous to use, as much of the flushing agent as possible should be shaken out.

Berliner (*8*) described a Mississippi model of artificial vagina built on the principle that the main stimulation of the ejaculation nerves is not brought on through the sphincter but by pressure of the vaginal walls against the end of the penis.

AT TIME OF WITHDRAWAL

This is the simplest method of collection, but the portion of the semen collected by this method is relatively low in spermatozoa count. By this method the male is permitted to serve the female in the usual manner, and as he dismounts the remaining semen is caught, in an enameled dipper, from the end of the penis. It should then be poured into a test tube or glass vial and covered. The receptacle, both dipper and container, should be warmed to body temperature, and in cold weather care should be taken to avoid sudden changes in temperature by wrapping the container in a towel.

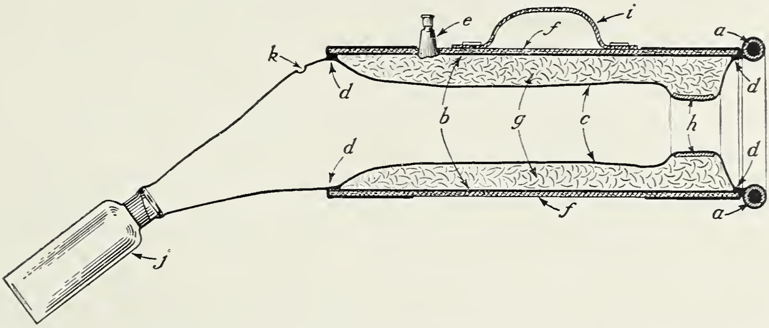

FIGURE 10.—Longitudinal section of the Missouri-U. S. D. A. model artificial vagina for the horse. Length 18 inches, 7-inch rubber tubing, flat diameter; *a*, Entrance ring made by enclosing section of ½-inch garden hose; *b*, outer tube; *c*, inner tube; *d*, points at which the outer tube is vulcanized to the inner tube; *e*, air valve; *f*, leather casing to give support and rigidity; *g*, air space between inner and outer tube which allows for the adjustment of pressure; *h*, the "sphincter" rubber band, made of 3-inch flat-diameter tubing 1½ to 2 inches broad; *i*, handle grip attached to leather casing; *j*, 8-ounce collecting bottle; *k*, air vent to prevent ballooning.

INSEMINATION

Stallion semen collected in the spring usually contains three fractions ejaculated in sequence. The first fraction (5 to 10 cc.) is watery, often grayish in color, and contains few or no spermatozoa. The second fraction (25 to 75 cc.) is thin or watery and contains 50,000 to 400,000 or more spermatozoa per cubic millimeter. The third fraction is viscous and, if present, may be the most voluminous, constituting 44 to 71 percent of the total volume of the ejaculate. Jacks have not been observed to produce much of the viscous fraction except in old age, and ordinarily stallions do not produce it in the fall. This viscous portion comes primarily from the seminal vesicles and the Cowper's glands and liquefies soon on standing. It is well to include it along with the high spermatozoa-containing fraction in the material placed in the mare at time of insemination.

The mare to be inseminated should be tried with the stallion to be sure that she is in heat. As a precaution against kicking, she should be hobbled, and if further restraint seems necessary, a twitch may be used or the foreleg held up. She should be stood facing a dark or

shaded wall with her tail to the light. The vulva should be wiped clean, then dried, and the inner lips swabbed.

A simple and convenient method of insemination is to use either a ½-ounce or 1-ounce gelatin capsule. From 10 to 30 cc. of semen (table 1) is poured into the capsule, which is then closed. It should be carried into the vagina without delay by the hand and placed well forward in the cervix. For this operation the hand and arm of the operator should be clean and well lubricated. Any rough or sharp edges of the fingernails should be removed. If a rubber glove and obstetrical sleeve are used in capsuling mares, infection can be kept to a minimum.

When an inseminator (syringe) is used for insemination, a speculum is first coated with a lubricant and introduced into the vagina by gentle rotation and pressure exerted in an upward direction until it passes over the brim of the pelvis, care being taken to avoid injury to the urethral meatus during insertion. The cervix should be clearly visible. An assistant should rinse the syringe with a little of the seminal fluid and then fill it completely with this fluid. All bubbles in the syringe and nozzle should be eliminated. This may be done by holding the syringe perpendicularly and forcing the bubbles out by a gentle upward pressure on the plunger. The nozzle of the syringe is then carefully inserted into the cervix and the semen slowly injected. From 10 to 30 cc. of semen should be injected, the amount depending on the number of mares to be inseminated and the quantity of semen available. A danger in the use of the speculum is that it always exposes the vagina to infection with bacteria or other microorganisms carried in the air.

Insemination may be made also by putting the arm into the vagina and inserting the tip of a rubber catheter into the cervix. The semen is injected by means of a syringe attached to the outer end of the catheter. The assistant should fill and operate it in the manner described in the previous paragraph. All air bubbles should be expelled before the catheter is introduced into the vagina. The operator gently works the tip of the catheter into the cervix. In mares fully in heat this is rather easily done, but if difficulty is encountered owing to rigidity of the cervix this usually can be overcome by gently massaging the cervix for a few minutes. This method may also be used effectively for collecting the semen from the vagina of a mare and introducing it into the cervix of the same mare following natural mating. The practice is sometimes successful in mares that are difficult to settle. However, it is unusually hazardous because of the difficulty in keeping this type of equipment clean.

If possible, mares should be inseminated near the end of the heat period since ovulation occurs about 24 hours before heat ends. The heat period normally is long, ranging from 3 to 7 days and varying from 1 to 15 days or more. It is often difficult, therefore, to tell just when heat begins, and it is sometimes desirable to inseminate two or more times during the period. Such practice is especially desirable in females that have extra long heat periods and are difficult to get settled. Breeding the third, fifth, and seventh days, if the mare is still in heat, is considered good practice as the chances for inseminating at the proper time are greatly increased.

Cattle

The procedures to be followed in the collection of semen and insemination in cattle are similar to those in horses. The apparatus needed depends somewhat on the method of collection. Following is a complete list of equipment needed for collection and insemination regardless of the method used:

Two small glass funnels, preferably of Pyrex. (Only one is needed, but it is best to have an extra in case of breakage.)

A test-tube brush with medium-soft bristles.

A 2-cc. graduated glass syringe.

A heifer-size speculum (when vaginal method is used).

An 18-inch ebonite nozzle equipped for attaching to the syringe. A glass tube with 1/8-inch inner diameter may be substituted for the nozzle. The ends should be well rounded in a flame and connected to the 2-cc. glass syringe by short pieces of rubber tubing (fig. 14).

An artificial vagina (Swedish or Russian type) and one spare inner tube. (This equipment is needed if collection is made by the artificial vagina.)

If the semen is to be collected by means of massage, the bull should be tied in a stall. However, if it is to be collected from the vagina of the cow or by use of the artificial vagina, the bull is handled as for normal mating. He should be led up to the cow with a staff or halter, depending on his disposition. The safety of the operator always should be assured. Information regarding the paddock to be used is given on page 7.

For vaginal collections, the cow may or may not be in heat, but she should be free from infectious diseases. In no case should a cow that has just calved be used. She may be tied, held with a halter, or preferably placed in a breeding rack or chute (fig. 11). The region around the vulva should be washed well with warm water and soap, a disinfectant, then rinsed with clear water, and the lips of the vulva spread and swabbed with clean cotton.

Hart and associates (*32*) found that some bulls that are consistently slow in mounting and yield poor-quality semen could be stimulated to vigorous activity and the ejaculation of high-quality semen through certain changes. Some responded when a new cow was substituted for the regular cow used for mounting; others, when a cow in heat was substituted. A favorable response was obtained by smearing the vulvar region of a cow out of heat with urine and vaginal mucus discharged from a cow in heat. At the Animal Disease Station, Beltsville, Md., a young bull that had never served a female was reluctant to mount a dummy cow and consistently yielded ejaculations almost devoid of spermatozoa. At the end of a year's collection he was turned in with a group of open cows. With few exceptions these cows settled at first service. During this time ejaculates collected at the time a cow was in heat were of excellent quality. Two bulls reluctant to serve the artificial vagina by mounting the regular female in stocks readily mounted a female in heat that was turned loose in a lot.

The psychic factor varies among individuals and may be the reason for a greater turn-over of bulls of some breeds than of others, for artificial insemination purposes. Bulls with good breeding history when leased by cooperatives may soon become poor breeders but again become satisfactory when returned to natural service. In the United States it is generally considered that the average usefulness of proved

bulls for artificial insemination is about 12 months, whereas of young bulls it is about 24 months. Bull-stud operators have found that some bulls will use the artificial vagina readily and consistently produce good semen for several years. However, collections from many bulls are either small or of poor quality or a combination of both.

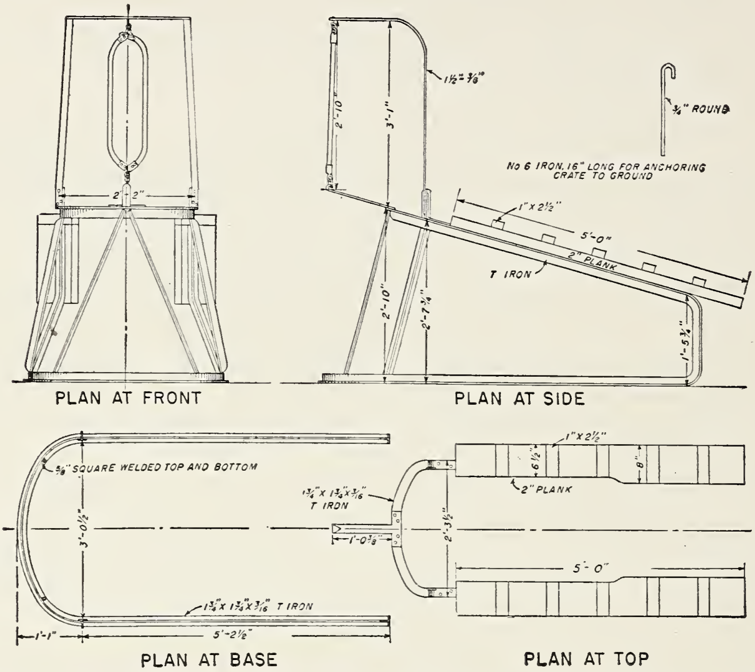

Figure 11.—Detailed plans of a good movable breeding crate for cattle. (From Edwards and Walton, courtesy Empire Journal of Experimental Agriculture.)

COLLECTION OF SEMEN

BY MASSAGE

Semen may be collected directly from the bull by massage of the ampullae of the vasa deferentia, but if this method is to be used a knowledge of the bull's anatomy and instruction in the technique are necessary. Details of the method, which was devised by Miller and Evans (*49*), are as follows: The bull is tied in a stall in such a manner that he cannot shift from side to side, and the sheath is carefully washed with a soft brush and warm water. This usually stimulates urination, which is desirable as it helps prevent contamination of the semen with urine. A rubber glove is placed on the hand and well lubricated. The gloved hand is then inserted in the bull's rectum a distance of 7 to 10 inches and the seminal vesicles (fig. 12, *A*) massaged by backward strokes and their contents, a turbid fluid containing relatively few spermatozoa, thus expelled. After massage of the

seminal vesicles the ampullae (widened ends) of the vasa deferentia (fig. 12, *B*) are massaged in a similar manner. The fluid from the latter ducts is very rich in spermatozoa. Miller and Evans reported 81 successful collections from 100 massages on 15 bulls. In none of them was there apparent injury. The quantity of fluid collected from the seminal vesicles at one massage ranged from 0.5 to 21 cc. and that from the ampullae from 0.5 to 23 cc. The small quantities were thought to be caused by the emptying of the ampullae shortly before they were massaged. Sometimes the ejaculate is retained in the sigmoid flexure of the penis. To avoid this, the operator should straighten this flexure with his other hand after massaging the ampullae.

The semen is collected from the end of the penis or sheath by means of a funnel and test tube held by an assistant, and after collection it is handled in the same manner as semen collected by other methods.

FROM THE VAGINA

This is the simplest method of collection. The semen is collected from the floor of the vagina, following copulation, by a long-handled vaginal spoon (fig. 13). Semen may be withdrawn from the vagina also by means of a syringe and nozzle (fig. 14). Although this may be done without using a speculum, its use facilitates more complete collection of the ejaculate, there is less chance of injury to the cow from insertion of the nozzle (or glass tube), and the chances of the cow's breaking the tube by sudden movement are minimized.

BY THE FISTULA METHOD

In a description of the collection of semen by means of a permanent perineal urethral fistula, Rowson (*71*) states that certain natives make an incision into the urethra of the stallion and then use him as a teaser, the sperm escaping by way of the fistula.

In his operation on a bull, Rowson inserted into the urethra, about 8 inches below the anus, a nickel-coated brass cannula shaped like a letter "J" to be kept in position as a permanent fixture. Blood clots had to be removed from the tube, and granulation tissue was a constant threat to closing the lumen of the cannula.

Such an operation performed successfully would be a great asset to artificial insemination in the following respects: (1) Bacterial contamination from the sheath would be prevented, (2) bulls that produce poor-quality semen because of their dislike for the artificial vagina would be likely to ejaculate normal samples, (3) semen could be collected from valuable bulls that refuse to use the artificial vagina, and (4) it would simplify the handling of the bull, which is one of the most difficult factors encountered in artificial insemination.

BY THE ARTIFICIAL VAGINA

The artificial vagina (figs. 14 and 15) should be filled with water heated to about 54° C. while held in a vertical position. The stopper is then inserted and the opening of the inner lining evenly and thoroughly smeared with a thin coat of lubricant.

FIGURE 12.—Median section of the bull showing the reproductive organs in position and the method of manipulating them for collecting semen by massage. *A*, Massaging the seminal vesicles; *B*, massaging the ampullae of the vasa deferentia. The organs are: *a*, Seminal vesicles; *b*, ampullae; *c*, body of prostate; *d*, pelvic urethra; *e*, bulbo-urethral (Cowper's) glands; *f*, urinary bladder; *g*, pubis. (After Miller and Evans.)

Limiting the lubricant to the first 6 inches of the liner helps to prevent bacterial contamination of the semen from the penis. The temperature may be determined by inserting a thermometer in the open end of the artificial vagina. It should not be above 44° nor below 40° when the apparatus is ready for use. If the water is too warm, time must be allowed for cooling; if it is too cold, some of it should be emptied out and replaced with hot water. Allowance should be made for a slight fall in temperature during the interval

FIGURE 13.—Vaginal spoon (Nils Lagerlöf type) used for collection of semen from heifers and cows. The spoon should be about 1 inch wide, 5 inches long, and the bowl ½ inch deep; the entire length, including handle, is about 30 inches. Preferably it should be made of ebonite.

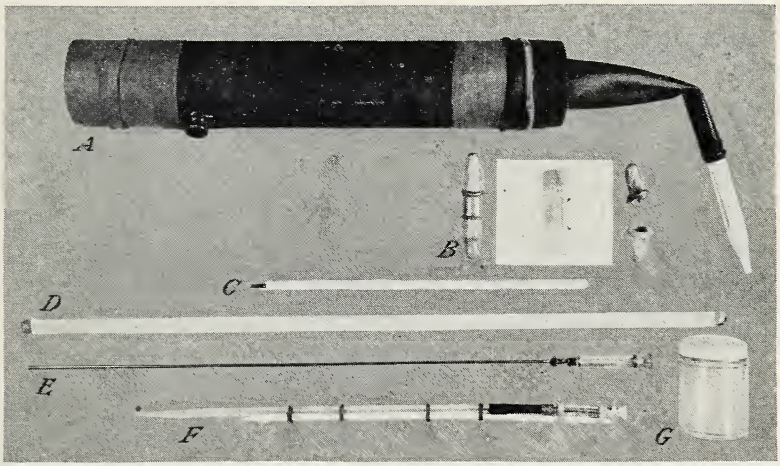

FIGURE 14.—Artificial-insemination equipment for cattle: *a*, Artificial vagina assembled for use (modification of Swedish model); *b*, semen vials with insulating paper and thumbstalls; *c*, thermometer, 0° to 100° C.; *d*, glass rod, 22 inches long, for applying lubricant to inner tube of the artificial vagina; *e*, rustless-steel inseminator and 2-cc. glass syringe attached; *f*, inseminator constructed of 2-cc. glass pipette connected with 2-cc. syringe by short piece of rubber tubing. Note that the apparatus is attached by rubber bands to a celluloid knitting needle to insure rigidity; *g*, wide-mouthed 4-ounce screw-top jar of lubricant. (Courtesy Missouri Agricultural Experiment Station.)

between the preparation of the apparatus and collection. After the proper temperature is attained the pressure must be adjusted. This may be accomplished by wasting some of the water in the artificial vagina, taking care to wipe the instrument dry thereafter. If the pressure is too great the collecting tube or vial may be forced out when the bull thrusts.

When the artificial vagina is ready, the bull is led up slowly behind the cow, and the collector follows the bull on the right side, grasping the apparatus in his right hand, the mouth being held downward.

When the bull mounts, the apparatus is inserted behind and to the outside of the bull's foreleg with the opening directed toward the penis at an angle of about 45°. The penis is directed into the opening of the artificial vagina by applying the left hand to the sheath. When the penis comes in contact with the warm lubricated surface of the artificial vagina the bull thrusts upward and ejaculates. Care must be used not to touch the penis as this may cause the bull to retract it and dismount. The semen is ejaculated into the upper end of the glass collecting tube or vial of the artificial vagina. Immediately the apparatus should be turned mouth upward to allow all semen to

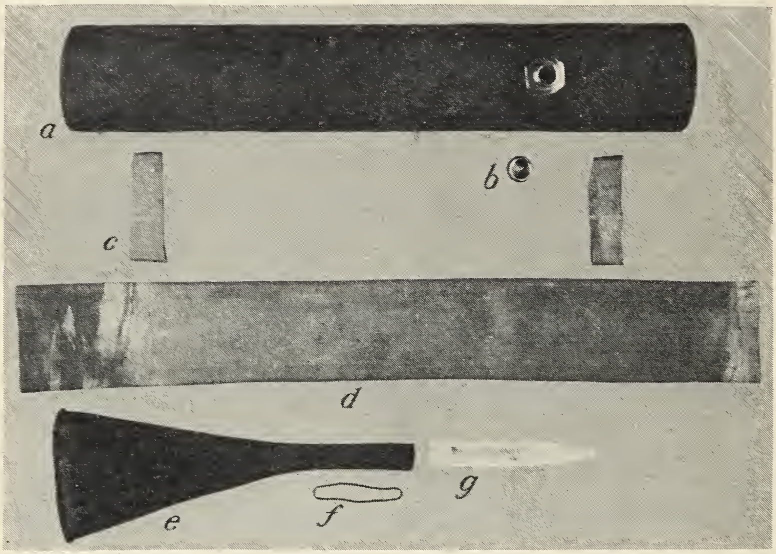

FIGURE 15.—Artificial vagina for cattle, unassembled; *a*, Stiff rubber casing 16 by 2¾ inches with brass valve; *b*, screw cap for valve; *c*, heavy rubber bands for securing each end of the inner tube to the outer casing; *d*, thin rubber tube, 20 inches long by 3 inches flat diameter; *e*, tapering thin rubber tube 10 inches long for connecting artificial vagina; *f*, small rubber band to secure tapering tube to glass tube or vial; *g*, glass collecting tube (8 to 12 cc.). This may or may not be graduated. If one wishes to use mineral oil in the glass collecting tube or vial a straight, flat-bottom vial or ordinary test tube must be used. (Courtesy Missouri Agricultural Experiment Station.)

flow into the tube or vial. The special procedures for handling semen have been described under handling and transporting semen after collection (pages 21 to 26).

INSEMINATION

The cow should be at the proper stage of estrus and confined in a stall or stanchion, preferably with a good light directly behind. If the light is insufficient, a head lamp may be used (fig. 17). The vulva should be wiped clean with cotton or washed clean and dried, as previously described.

Two methods of depositing semen in cattle are used in this country, namely, the speculum method and the cervical fixation or rectal

method. With the speculum method, the cervix is made visible at the end of the speculum. The point of the inseminating tube is then gently inserted from 1 to 2 cm. into the lumen (fig. 16), and the semen is expelled slowly into the cervix by gentle pressure on the syringe plunger. The semen should remain in the cervix and not run back into the vagina. Insertion of the speculum may cause the cow to arch her back and strain. This can generally be avoided or reduced to a minimum by using a speculum of small diameter.

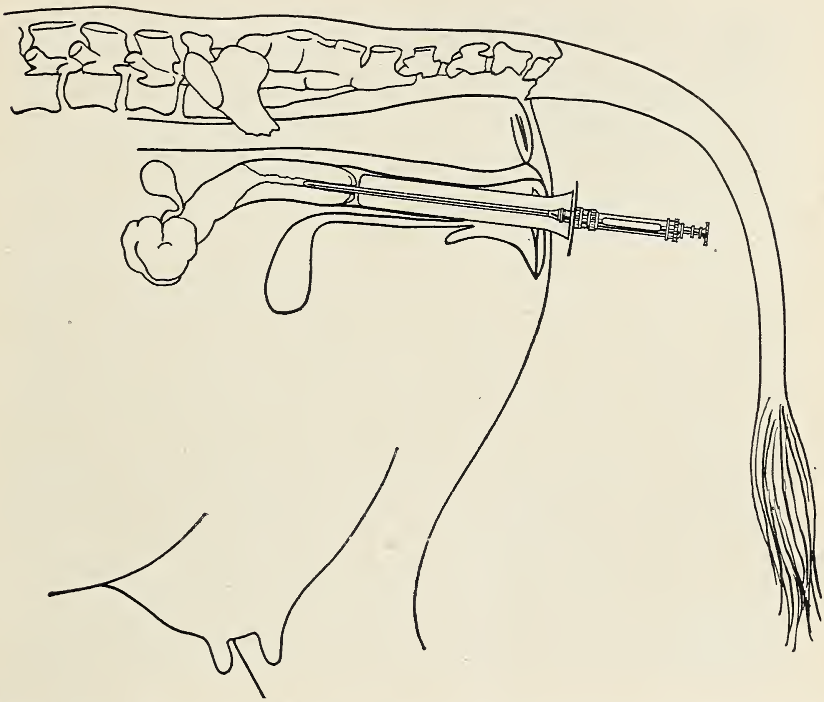

FIGURE 16.—Median section of the cow showing the reproductive organs in position with the inseminating syringe and tubular speculum in place. Nozzle of the syringe is shown inserted 1 to 2 cm. into the cervix.

In the cervical fixation or rectal method a gloved hand (obstetrical glove, shoulder length) is inserted into the rectum to guide the inseminating tube. The tube is inserted either one-half or two-thirds of the distance through the cervical canal or all the way through into the uterus. The conception rate in the deep cervical and intra-uterine deposition is approximately the same, and is equal to or slightly superior to the conception rate in the above speculum deposition. (See Place of Injection and Quantity of Semen, p. 32).

Sheep and Goats

The procedures in artificial insemination of sheep and goats are similar to those of cattle and horses. Rams and ewes, if the latter are to be used for collection, should be carefully selected, accustomed to their quarters and to handling, and attention given to those details

of management which will facilitate rapid mating with the production of copious ejaculates containing large numbers of spermatozoa. The equipment needed, most of which is shown in figure 17, consists of the following:

One 2-cc. glass syringe with glass plunger.

One ebonite or sterling-silver 10-inch nozzle. (10-inch glass tubes of ⅛-inch inner diameter that have been well rounded at one end and slightly tapered and rounded at the other may be substituted for the nozzle. These may be attached to the syringe by short pieces of rubber tubing.)

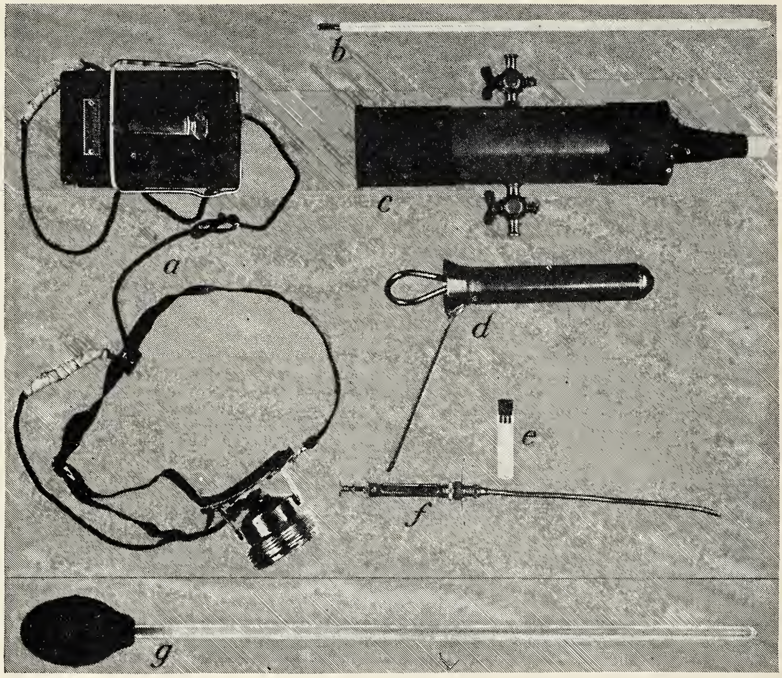

Figure 17.—Artificial-insemination equipment for sheep: *a*, Head lamp with battery; *b*, thermometer (0° to 100° C.); *c*, artificial vagina assembled for use (modified from Russian model); *d*, speculum 5 inches by ¾ inch; *e*, glass vial (2-cc.) for storing semen; *f*, inseminator consisting of 2-cc. syringe with glass barrel and 8-inch sterling silver nozzle (human urethral syringe; *g*, semen-collecting syringe for sheep consisting of a glass tube 12 inches long and a rubber bulb. (Courtesy Missouri Agricultural Experiment Station.)

A semen syringe (a human female urethral-type syringe) may be substituted for the first two items.

Small test tubes or bottles 2 to 5 cc. in size and preferably of Pyrex for holding semen.

A ewe-size speculum. (A Pyrex test tube 6 inches long with an inner diameter of three-fourths of an inch, the closed end of which has been cut off, makes a very serviceable speculum. The ends must be well rounded in a flame.)

A rubber tube with funnel or kettle with spout for filling artificial vagina with warm water.

An artificial vagina with two spare collecting vials and one spare inner tube.

When a ram is being trained it is advisable to select ewes that are in heat, and the ram should be allowed to serve a ewe several times

before an attempt is made to collect semen. Once a ram is trained, a ewe that is not in heat may be used.

A breeding rack mounted on a stand 18 to 20 inches high facilitates the collection of semen but is not essential. The advantage of using a rack is that it may be set at a convenient level for collecting semen either from the ewe or from the artificial vagina. If a rack is not used, the ewe should be placed in a stanchion, tied to a post or fence, or held by an assistant.

COLLECTION OF SEMEN

FROM THE VAGINA

A ewe not in heat should be used because the vaginal secretions are not so extensive and, consequently, the semen is obtained in a less contaminated state. Such a ewe may be used repeatedly for collection without any detrimental effects to her. The ram is led up to the ewe and allowed to copulate one to three times, after which he should be removed. The semen is collected from the anterior region of the vagina by means of the syringe (fig. 16) and placed in a test tube or other receptacle. See method of handling under Use of Diluters (p. 22).

BY THE ARTIFICIAL VAGINA

The steps in the preparation of the artificial vagina (fig. 16) are similar to those described for similar models used for the bull. The artificial vagina should be partly filled with water heated to 50° C. The temperature at the time of collection should be between 41° and 44°. If it is below 40° or above 45° it may inhibit ejaculation. Pressure in the artificial vagina, if too great, may be adjusted by opening a valve, or if too low, by blowing in more air through the valve. An approximation of the desired pressure may be determined by inserting the thumb into the lumen. It should pass easily, but a slight pressure should be felt. The pressure needed will vary from ram to ram, but with a little experience an operator will soon determine the proper pressure.

When the artificial vagina is ready the operator holds it in his right hand with the open end down at an angle of about 45°, and stands close to the right flank of the ewe. As the ram mounts, the apparatus is interposed behind the ram's foreleg with the opening directed downward toward the penis at an angle of about 45°. The penis is directed into the mouth of the artificial vagina by the left hand, which is applied to the sheath, care being taken not to touch the penis itself. When the penis touches the warm lubricated surface of the artificial vagina, the ram thrusts upward, and semen is ejaculated into the upper end of the tube and collected in the vial. As the ram withdraws, the apparatus is turned mouth upward to permit all the semen to drain into the vial. The vial is then removed and corked. Subsequent treatment of the semen is given under Use of Diluters (p. 22).

BY ELECTRICAL STIMULATION

Collection of semen by electrical stimulation, developed by Gunn (*26*), is effective with rams but, because of the expensive apparatus

and the care required in its use, it is valuable chiefly for collections made in the laboratory. The apparatus required (fig. 18) consists of a small transformer of about 2 amperes' capacity, a voltmeter and milliammeter, and two electrodes soldered to insulated wire leads. The alternating current from an electric plant may be rendered suitable by being passed through the primary of a small transformer and tapped in the secondary at voltages ranging from about 10 volts by fives to 40 volts. If an alternating current is not available the current from batteries of suitable voltages may be used, but such current must be passed through a small interrupter and reverser. The intermittent current of reversing polarity is then passed through the voltmeter and milliammeter before being conveyed to the animal. In order that the exact voltage and milliamperage of the current may be

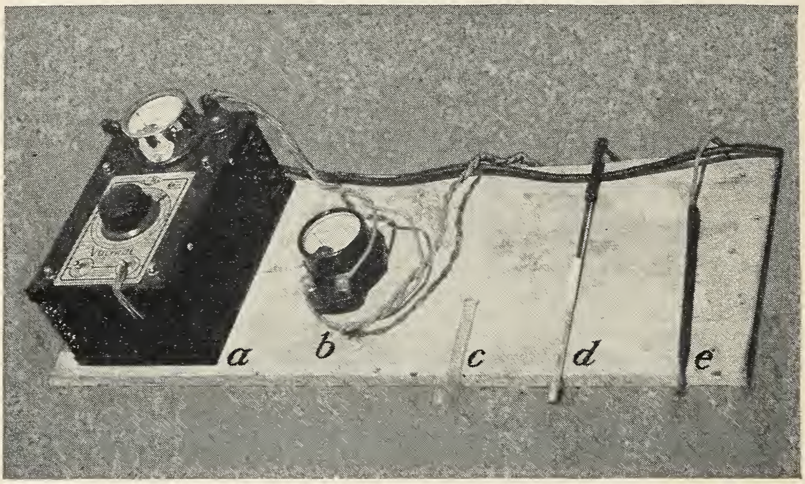

FIGURE 18.—Apparatus and method used for collecting semen by electrical stimulation at the United States Sheep Experiment Station, Dubois, Idaho. The parts shown are: *a*, Voltmeter; *b*, ammeter; *c*, collecting tube; *d* and *e*, electrodes.

determined before it passes to the animal, the leads are passed to a voltmeter and to a milliammeter before continuing to the animal. The electrode for insertion in the rectum consists of a smooth wire of about the diameter of an ordinary lead pencil; the other electrode consists of a wire flattened at one end so that it has a surface area of about one-fourth inch in diameter. Gunn used a stout needle as the second electrode, the needle being inserted in the longissimus dorsi muscle.

In experiments at the United States Sheep Experiment Station, an electrode applied tightly to the surface of the skin in the region of the fourth lumbar vertebra was as effective. To insure good contact the skin is first moistened in this region. Both electrodes should be well insulated at the point of soldering to the insulated wire leads in order to prevent shock to the attendant holding the electrodes in place. The apparatus should be placed on a small table about 2 feet high.

When the apparatus is in place and ready for use, the ram is led in, and laid on his side on a table or bench about 2 feet high. His fore-

legs are tied together and secured in a forward position (fig. 19). The hind legs are similarly secured and extended backward. The head also is fixed in an extended position. The feces should first be emptied from the rectum and the long electrode then inserted. The other electrode should be firmly held to the skin in the region of the fourth lumbar vertebra by an assistant. A series of 10 to 20 stimulations at 30 volts are then applied, the current being on 5 seconds and off 5 seconds. The number of stimulations given depends on the quantity of semen wanted.

When this method is properly used, repeated collections may be made from the same ram at daily intervals or on every second day over long periods without any harmful effects. The semen is normal in all respects, but in general the first portion of the ejaculate so obtained is thin and watery. The main bulk of the ejaculate containing

FIGURE 19.—Electrical equipment being used for collection of semen from a ram. Note ammeter and voltmeter, at right.

most of the spermatazoa is obtained from the second or third to the twelfth or thirteenth stimulus. Considerable variation is observed between different rams in the number of stimuli required to produce good ejaculation.

The semen is collected in a test tube. Before the collection is made, the penis should be washed with normal saline solution, and if the semen is to be in a near-sterile condition only the urethral process should be allowed to enter the neck of the collecting tube. Difficulty is sometimes encountered in getting the penis to extend, especially in young rams. To accomplish this, the sigmoid flexure should be straightened and the sheath then pushed to the rear. Wrapping a small piece of gauze around the end of the penis helps to keep it extended.

This method has a number of disadvantages: (1) The apparatus needed is expensive, (2) care must be exercised to control the degree of shock applied, (3) the semen is sometimes contaminated with urine, and (4) considerable skill is required on the part of the operator.

The chief advantages are: (1) The semen is obtained in a near-sterile condition, and (2) the method is effective for use in valuable males that cannot copulate normally because of injury, in males that have lost their sexual desire, or in males too young to have learned to copulate.

INSEMINATION

Insemination may be carried out most easily if the ewe is placed in a crate 18 to 20 inches off the ground and mounted on an axle that will allow it to be swung in front of the operator and so tilted as to lower the head of the ewe. If a crate is not available, the ewe may be stood on a table, or a small pit in which the operator can sit may be dug. The purpose is to bring the eyes of the operator on a level with the vulva of the ewe.

The syringe should be filled and made ready for introduction into the vagina. When the ewe is in position the vaginal speculum (fig. 16) is introduced into the vagina and the cervix brought into view with the aid of a head lamp. If a glass speculum is used, one end should be rounded somewhat to facilitate insertion. Before insertion the speculum should be well covered with lubricant. The tip of the nozzle on the syringe is introduced gently, by the right hand, about 1 cm. into the cervix and the plunger depressed until about 0.1 cc. of semen is injected. Inseminations should be made during the last half of estrus or at 12-hour intervals as long as estrus lasts.

If a number of ewes are to be inseminated at one time, they should be run into a pen near the place where inseminations are to be made. Careful attention should be given to the arrangement of apparatus and equipment to facilitate all operations. If it is necessary to use a dry, dusty place for the purpose, the ground should be well sprinkled before either collections or inseminations are undertaken.

SWINE

Although artificial insemination has not been practiced to any great extent in swine, enough experimental work has been done to demonstrate that the techniques are comparatively simple and that the results are satisfactory when inseminations are made at the proper stage of estrus. Semen collections are made by means of an artificial vagina, and the apparatus is only slightly different from the kind used for cattle, horses, and sheep. As the boar produces a large quantity of ejaculate, from 70 to 80 cc. per 100 pounds' weight, a large cup is necessary for the semen. Collections may be made with a relatively simple type of vagina in which no special provision is needed to keep the apparatus warm during copulation. It consists of a soft rubber tube (band tubing) 16 inches long and 1$\frac{3}{16}$ inches inside diameter and 1$\frac{1}{4}$ inches outside diameter, one end of which is fitted over a suction flask and the other end rolled over a 1$\frac{5}{8}$-inch key ring. A rubber clamp completes the outfit. Another type of artificial vagina resembles that used for cattle. It consists of an ebonite cylinder provided with valves for regulating the pressure and an inner rubber chamber. In addition there is a rubber tube 180 mm. long and 80 mm. wide which connects with a semen receptacle of 500 to 800 cc. capacity. Baeckström, of Sweden, uses one similar to that described above for cattle, except that a bulb is attached to an air line to give pulsations.

Another type improvised at the Missouri Agricultural Experiment Station by Lasley and McKenzie consists of an inner tube, 1⁵⁄₁₆ inches in inner diameter and 1³⁄₈ inches in outer diameter, and an outer rubber casing 12 to 15 inches long (fig. 20, *B*). The semen is collected in any convenient glass receptacle, preferably 50-cc. test tubes.

COLLECTION OF SEMEN BY THE ARTIFICIAL VAGINA

When the operator is about ready to make the collection, the inner surface of the tube is evenly coated with lubricant and the whole apparatus (simple type previously described) immersed in water heated

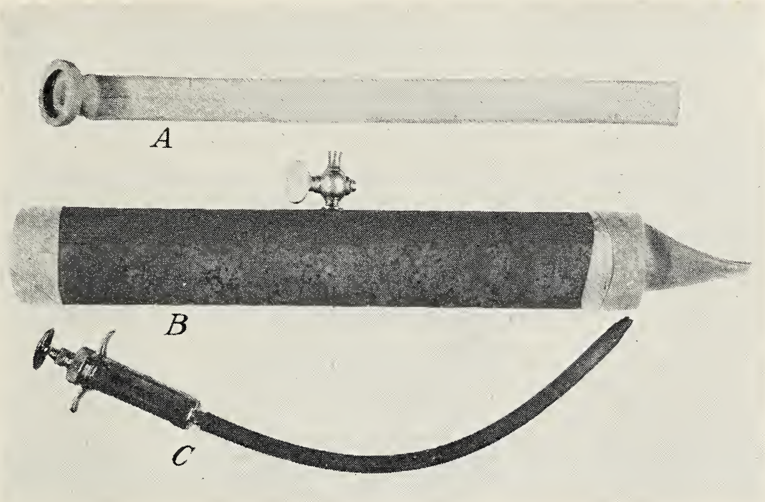

FIGURE 20.—Artificial insemination equipment for swine: *A*, A simple artificial vagina consisting of thin rubber tubing 16 inches long and 1¼ or 1⅜ inches flat diameter with a 1⅝-inch key ring in one end; *B*, another type of artificial vagina consisting of thin rubber tubing similar to the above, together with a rubber casing 12 to 15 inches long and 1¾ inches in diameter. One air valve permits adjustment of air pressure; *C*, inseminator consisting of 50-cc. syringe with glass barrel, and 18-inch rubber pressure tubing, inside diameter ³⁄₁₆ inch, outside diameter ⁹⁄₁₆ inch.

to about 45° C. if the apparatus is to be used in cold weather. Care must be used, however, to avoid getting water into the flask used for collection. A sow is placed in a simple stanchion or tied to a wall by means of a rope around her upper jaw and the boar admitted. When the boar mounts and attempts to copulate, the open end of the tube is placed in front of his sheath so that the penis can pass into the tube. As the penis enters the tube it is manipulated with a pulsating motion by the attendant's hand, thus encouraging continued copulation and ejaculation. Too much pressure must not be applied against the sheath or a part of the contents of the preputial diverticulum may be forced into the collecting flask. The semen is collected in the flask, and after the boar withdraws the clamp is placed on the tube just above the flask. From 5 to 20 minutes is required for collection, depending on the condition and disposition of the boar.

The Swedish model vagina is used in much the same way. A pulsating motion on the penis may be obtained by means of a rubber bulb. The recent Missouri model (fig. 20, *B*) is simple and satisfactory. No water or heat is required, and the correct pressure is adjusted through the air valve prior to use. Pulsations on the penis are made by frequently squeezing the casing. Otherwise the instrument is lubricated and handled in the same manner as other models.

Spermatozoa are not ejaculated uniformly by the boar throughout the period of copulation, according to McKenzie, Miller, and Bauguess (*44*), but appear in waves of great concentration at certain intervals when the rate of discharge of semen is greatest, usually the second to fourth minute of ejaculation. It is well, therefore, to mix the ejaculate well before inseminations are made and to remove the gelatinous lumps. Not more than one service a day should be allowed, and if the boar is being used regularly there should be 1 day of rest following 2 days of service. With yearlings, 2 days of rest between copulations is sufficient to restore both the total volume of semen and the number of spermatozoa, whereas with 1 day of rest the volume is restored but the number of spermatozoa remains somewhat reduced.

Sexual attraction plays relatively little part in the mating behavior of a boar, so it is usually unnecessary to have a sow in heat. Boars, likewise, may be readily stimulated to mount a dummy sow. The quarters used for collection should allow plenty of space for turning and should be equipped with gates to permit easy admission and removal of the animals, and the floors should be of such construction that the danger from slipping is minimized.

INSEMINATION

The structure of the genital tract of the sow is such that only simple apparatus is required for insemination. The following conditions insure that fertilization will take place following normal mating: (1) The semen is ejaculated directly into the cervix by the corkscrew-shaped glans penis, (2) the volume of semen is very large, and (3) a vaginal plug forms after withdrawal of the penis. The plug is composed of the last fraction of the ejaculate and consists of a stiff waxy material secreted almost entirely by Cowper's glands. In artificial insemination it is desirable that these conditions be simulated. From 50 to 100 cc. of semen should be placed well in the anterior end of the vagina. The gelatinous portion of the semen should be discarded before the syringe is loaded. This can be done by separating out these lumps and ejecting them from a glass bowl by a glass rod or by straining the semen through freshly laundered cheesecloth. If the fraction containing the high concentration of spermatozoa is used, only about 25 cc. mixed with a good swine diluter (page 23) may be necessary. After the semen is deposited in the anterior end of the vagina of the sow, it is well to push a moistened cotton plug just into the vestibule of the vagina. This prevents excessive waste of semen.

Because of the large uterine horns in the sow, relatively large volumes of semen are sometimes recommended for insemination. No conclusive data are available as to the quantity; but, with undiluted semen, volumes of 50 to 100 cc. seem sufficient if the concentration of spermatozoa in the semen is reasonably high. If the semen is diluted

four times, the optimum volume is 100 to 150 cc. depending on the size of the sow.

Before the sow to be inseminated is led in, all apparatus should be made ready and placed in a convenient location for the operator. The sow should be placed in a crate or breeding chute or tied to a wall by means of a rope around her upper jaw and should, of course, be at the proper stage of estrus (table 3). The equipment needed consists of a glass syringe of 50 cc. capacity and one piece of rubber pressure tubing 45 cm. long and of about 4 mm. inner diameter that has been fitted to the syringe and tapered on the free end (fig. 20). An ebonite nozzle attached to the syringe may be substituted for the rubber tubing. When the sow is ready for insemination and the syringe and tubing (or nozzle) have been filled with semen, the nozzle is introduced into the vagina and forward into the cervix. The semen is slowly expelled into the cervix by pressure on the plunger of the syringe.

Dogs

Although the dog was one of the first animals used for the study of artificial insemination, relatively little recent experimental work has been done with this species. The techniques of collection and insemination, however, are relatively simple and the method may prove of distinct value to dog breeders.

COLLECTION OF SEMEN

With most dogs collection of the semen is easily made by manual manipulation of the penis. The dog is placed on a table or in a rack at a convenient height from the floor, and the base of the penis is manipulated with the hand until erection is induced. The prepuce is then pushed posterior to the bulbous portion of the penis so that erection is maintained. Under such conditions the accessory glands show irritation and superfunction; ejaculation occurs when the penis is touched. The semen is collected in a clean, dry porcelain or glass receptacle, a 25- to 50-cc. test tube or vial being of convenient size. The semen should then be set in a cool, dark place until it is used. If inseminations are to be made immediately after collection, the semen may be collected directly in the glass syringe used for inseminations by removing the plunger and keeping the finger over the shoulder end of the syringe to prevent escape of the semen. The volume collected varies with the size and condition of the dog, but with medium-sized dogs the volume averages about 7 cc. Frequent collections may be made without any ill effects although not oftener than once in 2 days if a dog is in regular use for this purpose. Otherwise the spermatozoa count of the semen will be lowered.

Semen may also be collected by an artificial vagina. This method is preferable when studies are to be made on physiological problems concerned with ejaculation and spermatozoa production since it simulates normal coitus closely.

INSEMINATION

Inseminations are made by means of a 20- to 30-cc. glass syringe and a small rubber catheter (cat size) attached to the syringe. The

female to be inseminated should be in the proper stage of heat, preferably the eleventh to thirteenth day after beginning to bleed. She should be placed on a table or in a rack and restrained in such a manner as to prevent sudden or excessive movement. The rack should be high enough from the ground to bring the vulva on a level with the eye of the operator. All equipment should be made ready and placed conveniently for the operator before insemination is undertaken.

The vagina is opened by means of a small speculum; a short test tube of ½-inch inner diameter, the closed end of which has been cut off and the ends well rounded in a flame, does very well. The tip of the catheter is placed directly in the cervix and the semen slowly expelled by gentle pressure on the plunger of the syringe. The female should be placed so that the vulva is toward a bright light or, preferably, the operator should wear a head lamp. This permits direct observation of the cervix during the course of insemination, and the chances of injury to the female are minimized. After the semen is injected, a moist cotton plug is inserted just inside the vulva and the hind quarters kept elevated for 10 to 15 minutes to prevent as little loss as possible of the semen.

FOXES [10]

Although artificial insemination offers great promise of increasing the use of valuable male foxes, relatively little experimental work has been done with this species. Hence entirely adequate techniques have not been developed, although some success has been obtained with the techniques described below.

COLLECTION OF SEMEN

A method of collecting semen from fur animals by electrical stimulation has been reported recently by Dalziel (*23*). One that has been used for some time in foxes with satisfactory results, and that is similar to the method used in sheep, is as follows.[11] Previous to collection the fox is restrained and laid on his side on a table of convenient working height, about 30 inches, and one electrode is inserted about 3 inches in the rectum. The other electrode is placed between the fourth and fifth lumbar vertebrae. In order that the exact spot for placing the electrode may be located, an area approximately 1 inch square should be sheared. As a source of current in experiments conducted in the United States Department of the Interior, a laboratory magneto mounted in such a way that it could be operated by hand crank was used. It delivered 35 volts and 20 milliamperes at approximately 30 revolutions per second. The current was applied for 5 seconds and then was cut off for 5 seconds. Ten to twelve shocks were usually sufficient to produce ejaculation. In the early experiments considerable difficulty was experienced owing to contamination of the semen with urine, but with improvement of the technique this difficulty was largely obviated.

[10] Frank G. Ashbrook of the Bureau of Biological Survey, U. S. Department of the Interior, and Charles E. Kellogg of the Bureau of Animal Industry, U. S. Department of Agriculture, assisted in the preparation of the sections dealing with artificial insemination of foxes and rabbits.

[11] R. T. Clark of Montana State College, Bozeman, Mont., described this method in a personal communication.

Copper electrodes 4 to 6 inches in length were used, but the electrode to be inserted into the rectum was covered with glass tubing except for the tip, which was flared back over the tubing and then smoothed to prevent injury to the fox. The glass served as an insulator and permitted contact within the fox only at the extreme tip of the electrode.

With this method from ½ to 1 cc. of semen was obtained from a fox at each collection. This is a somewhat smaller volume than was reported by Starkov (*78*), who gives 1.5 cc. as the average volume of the ejaculate obtained from foxes by mechanical manipulation. The range in volume of semen reported by Starkov was 0.1 to 4.5 cc. He states that semen can be easily obtained from foxes by this method. In the hands of workers in the Department of the Interior, however, this method has not proved successful.

INSEMINATION

The technique of insemination used in the bitch is satisfactory also in the vixen. Mating usually takes place toward the end of estrus, namely, about 6 days after the beginning of heat, and inseminations should be made, according to Starkov (*78*), at about this time. However, considerably more experimental work is needed on the estrual cycle in the vixen before final recommendations can be made about the optimum time for insemination. Starkov states that the syringe should be retained in the vagina for some time after insemination to prevent wastage of semen. This difficulty could probably be obviated by placing a cotton plug moistened with saline solution just within the vulva.

Since adequate methods of storing the semen of foxes have not yet been devised, inseminations should be made as soon as possible after collection.

Rabbits

The rabbit has proved to be an excellent experimental animal for studies on the physiology of reproduction, as the doe ovulates only after copulation or after sexual excitement produced by does mounting each other and at a rather definite interval thereafter, namely, about 10 hours (fig. 6). As a result this species has been used extensively in studies on artificial insemination.

COLLECTION OF SEMEN

Collection by means of an artificial vagina is the most satisfactory method with rabbits. The artificial vagina, devised by Macirone and Walton (*42*), of England, consists of a glass tube or vulcanite cylinder closed at one end by means of a tapering inner rubber sleeve, which is turned back over the edge of the tube and held in place by rubber bands. The rubber sleeve extends through the glass tube and through the center hole of a rubber stopper, which closes the other end of the artificial vagina. The semen is collected in a small glass vial inserted through the center hole of the rubber stopper and on the inside of the rubber sleeve (fig. 21, *A*). Water is introduced into the cylinder and the pressure regulated by means of two glass tubes that pass through the rubber stopper and are fitted with rubber connections. The cylinder is filled with warm water heated to about 45° C., and approxi-

mately the right pressure on the rubber sleeve is obtained by holding the tube upright and filling it with water to the level of the tube labeled *g*, in figure 21, *A*. The water is retained in the vagina by clamps on the rubber tubing (*h*). Before being used, the inner surface of the rubber sleeve should be smoothly coated with lubricant. Following service the open end of the vagina is turned upward to allow the semen to collect in the container at the inner end of the tube. As with all equipment used for the collection of semen, precautions must be taken to see that the apparatus is clean and dry before it is used, and allowance should be made for a slight drop in the temperature of the water between the time of filling and the time of use. After collection the semen should be set aside in a cool, dark place, and handled in a way similar to that described for other species.

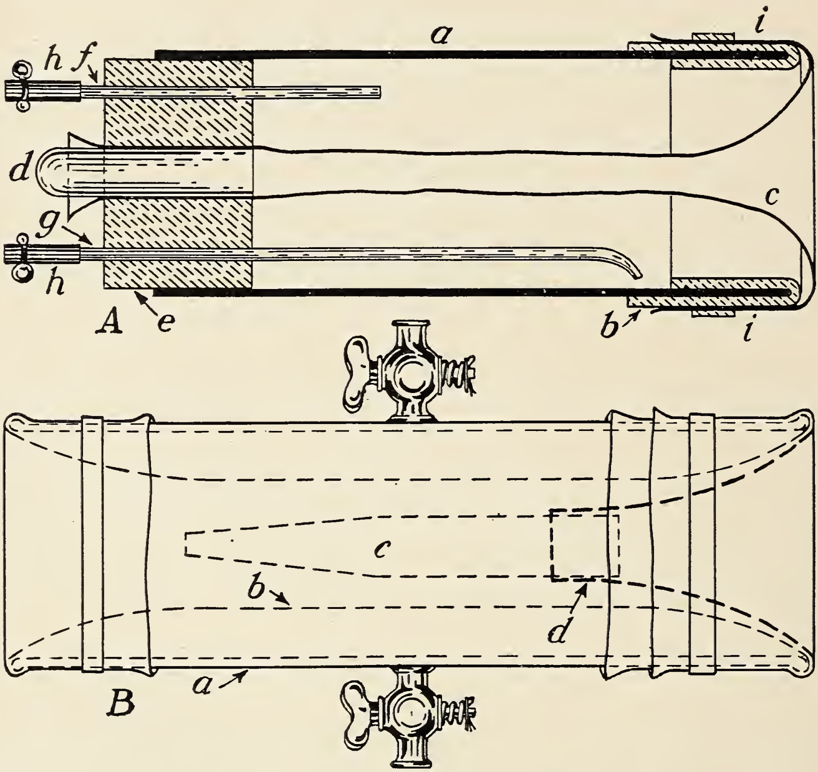

FIGURE 21.—*A*, Longitudinal section of artificial vagina for the rabbit, about actual size: *a*, Glass tube or ebonite cylinder about 1¼ inches in diameter and 3¼ inches long; *b*, thick rubber tubing covering the edge of the cylinder; *c*, thin inner rubber tube or sheath 5 inches long, 1³⁄₁₆ inches in diameter at one end and tapering to ½ inch at the other; *d*, glass specimen tube or vial which fits inside the sheath and holds it in position; *e*, rubber stopper; *f* and *g*, glass tubing for the introduction of water; *h*, rubber connections and clamps; *i*, rubber band to hold the inner sheath in place. (After Macirone and Walton, Journal Agricultural Science, slightly modified.) *B*, Longitudinal section of artificial vagina for rabbit (Missouri model): *a* and *b*, Outer casing and inner tube, respectively, of the sheep artificial vagina; *c*, 10-cc. centrifuge tube; *d*, rubber thumbstall.

Recently Cooksey and McKenzie at the Missouri station have developed an artificial vagina for the rabbit that is simple to make and to operate. The artificial vagina designed for sheep (fig. 16, *c*) contains a 10-cc. centrifuge test tube. A fingerstall is pulled over the mouth of this tube and is attached to the cut-off end of the tube by means of rubber bands. The open end of the fingerstall is stretched and reflected over the end of the sheep artificial vagina. Thus the water jacket of the larger sheep vagina provides adequate heat and pressure, and the fingerstall adapts the instrument to the rabbit (fig. 16, *b*).

The artificial vagina, when ready for use, is held between the hind legs of a doe which has been placed in the buck's cage. When the buck mounts the doe her hind quarters are lifted by the artificial vagina in the operator's hand, and the buck's penis enters the lubricated, open end of the artificial vagina. With a little experience in handling the instrument, collections can be made with any buck that is accustomed to the presence of the operator. Once a buck is accustomed to use the artificial vagina, collections may be made with a dummy. This consists of a glove made of rabbit skin, fur out, which is worn by the operator on the hand in which he holds the artificial vagina.

With the artificial vagina repeated collections may be made from vigorous bucks, and semen in quantities varying from 0.5 to 6.5 cc., in exceptional cases, is obtained. Before the semen is used for artificial insemination, a sample should be checked for sperm concentration, motility, and abnormalities.

Males vary greatly in their sexual desire and, therefore, in the readiness with which they will use the dummy or even copulate with a doe. In general it is best to introduce the female into the cage of the male, for most males respond best in familiar surroundings. Once a male becomes accustomed to serving females introduced into his quarters, it is usually easy to substitute the dummy for the female and induce the male to mount.

Semen may be collected also directly from the vagina of the female, after copulation, by aspiration with a small syringe that is inserted into the vagina. If this method is used, the doe should be grasped by the hind legs by an assistant and held firmly with the head down. Care must be used in inserting the syringe to avoid injury to the female. The disadvantages of the method are that the yield of semen is small, the semen is mixed with vaginal secretions, and there is danger of spreading infection.

Another method for the collection of semen consists in inserting in the vagina a small sponge to which a stout thread is fastened. Following copulation, the sponge is removed and immediately squeezed and rinsed in a small quantity of Ringer's solution to remove the semen. The disadvantages of this method are that it is difficult to get good copulation, the yield of semen is small, there is the chance of spread of infection, and many spermatozoa are injured as they are squeezed from the sponge.

INSEMINATION

Two attendants are required for insemination. When all apparatus is ready an assistant grasps the doe and holds her between his knees,

head down and with the back toward him, with the hind legs held uppermost and slightly apart, one in each hand. The second man then inserts the small glass tube of semen into the vagina, taking care to pass it over the pelvic brim before squeezing the bulb and expelling the semen. The recommended volume for the doe is 0.25 to 1.0 cc.

Normally the doe ovulates only after copulation, or sometimes after sexual stimulation due to the mounting of other does, approximately 10 hours afterward. Consequently if many inseminations are to be made, one or more vasectomized bucks should be on hand. These bucks, allowed to copulate with the female, stimulate ovulation. The English physiologist, Hammond (*29*), has shown that mating should occur from 5 to 10 hours before ovulation if maximum fertility is to be attained (fig. 6). The drop in both number of fertile matings and the number of young per mating was very rapid when the interval between copulation and ovulation was less than 5 hours. Since the doe normally ovulates 10 hours after coitus, insemination should be made within 2 to 5 hours after mating with the vasectomized buck has occurred. If vasectomized bucks are not available, a normal buck may be allowed to mount the doe, but in such a case an apron should be interposed to prevent the insertion of the penis into the vagina. Ordinarily this is sufficient to stimulate ovulation.

Chickens and Turkeys

Although artificial insemination has been practiced relatively little in birds, recent techniques have been developed that make it useful and practical in certain phases of poultry husbandry. It makes possible a greater use of progeny-tested males, is a valuable means of bringing about fertility of hens kept in batteries, crosses between species, or crosses between breeds that differ greatly in size. For studies on certain aspects of the physiology of reproduction it is also an invaluable tool.

COLLECTION OF SEMEN

A very practicable and easy method of collecting semen from the cock and turkey tom has been described by Burrows and Quinn (*19*). The method, which involves manual manipulation, requires two operators. The bird is held loosely by the thighs by one operator, who supports as much of the bird's weight as possible by extending his fingers under the breast. The rear of the bird is toward the second operator, and the legs of the bird are spread slightly apart so that the abdomen is well exposed. To obtain semen, the second operator causes the copulatory organ to protrude slightly from the vent by rapidly massaging the soft part of the abdomen, while the tail of the bird is forced upward over its back with the heel of the left hand. The thumb and forefinger of the left hand are held in readiness to grasp the vent from above to force the copulatory organ outward as soon as it can be seen protruding from the vent. The semen is expelled into the receiving container, which is held in the right hand of the second operator, by a slow milking manipulation of the copulatory organ. The various procedures involved in collection and insemination are shown in figures 22 and 23.

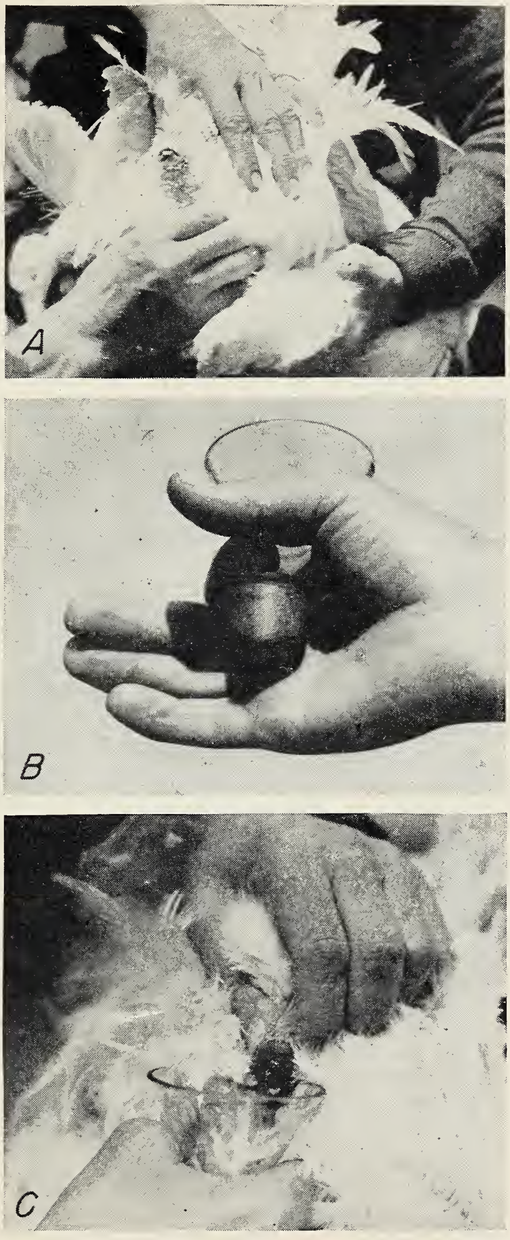

FIGURE 22.—Procedure in the collection of semen from the cock: *A*, Stimulation of the fowl (the operator's left hand is used here only to expose the field for the camera); *B*, container for collecting semen being held so that the fingers are free for stimulation; *C*, showing the left thumb and forefinger holding the copulatory organ exposed. (From Burrows and Quinn.)

With this method of collection it is very important that the male be held loosely, for gripping him tightly inhibits the desired reactions. Continued rapid massage of the abdomen following collection causes the bird to go through an ejaculatory response that assists in refilling the ducts for repeated collections of semen. A bird may be milked two to six times at each operation, or as long as semen is obtained. It is not necessary to attempt to obtain an ejaculatory response for repeated collections in turkeys. The cock will usually produce 0.2 to 2.0 cc of semen per day and the tom 0.1 to 0.8 cc., although most toms produce 0.3 to 0.4 cc. per day. Semen should not be collected oftener than once a day.

FIGURE 23.—Steps in collecting semen and inseminating the turkey: *A*, Method of holding the tom; *B*, collecting the semen, the copulatory organs exposed; *C*, holding the female for insemination; *D*, method of inseminating the hen.

A small 60°-angle funnel is used for collection. A one-hole, rubber stopper should be fitted to the funnel to serve as a grip and the stub end below this cut off and the opening filled with paraffin (fig. 22, *B*). If the operation is properly carried out, collection may be made with very little soiling. When contamination with feces occurs, it is obvious because of the discoloration, but contamination from urine may not be so obvious. When collections are being made from more than one male, the semen should be transferred, as collected, from

the receptacle used for collection to a test tube so that contamination, if it occurs, will ruin but one sample.

Males vary greatly in their response, and some difficulty is usually encountered in making the first collection from a male. In learning the technique it is well to try a number of birds until one is located that responds readily. After the technique is learned on such a bird, collections from other males will be easier. Males kept in batteries or pens apparently produce best. Only an occasional male is found from which semen cannot be obtained by this method.

Parker (*57*), at the Missouri station, has devised a simple technique of collecting semen from the cock. The area surrounding the vent is plucked, wiped with 70-percent alcohol, and a glass cup 2½ inches

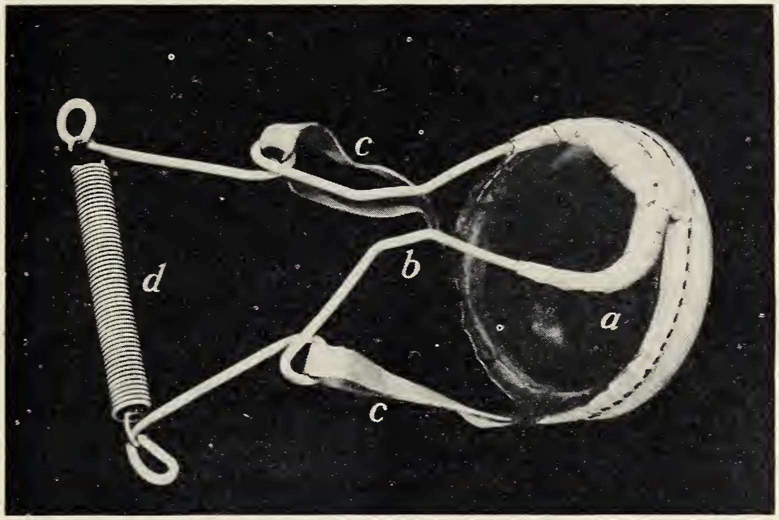

FIGURE 24.—Device for collecting semen from the cock: *a*, Glass receptacle 2½ inches in diameter and 1 inch deep; *b*, wire clamp; *c*, rubber bands; *d*, No. 14 bronze-wire spring. (Courtesy Missouri Agricultural Experiment Station.)

in diameter and 1 inch deep, which has been coated with paraffin, is held over the vent by means of a clamp over the tail and a No. 14 bronze-wire spring across the back (fig. 24). Elastic bands hold the cup firmly over the vent. The bird is then turned loose among some pullets to tread. He ejaculates into the cup, and the semen is withdrawn from the cup by means of a small pipette or medicine dropper and transferred to a vial. Another model has a removable glass vial attached to the collecting cup, so attached that the semen flows into the vial. This type of cup is advantageous if one finds it necessary to collect semen frequently.

INSEMINATION

The method of inseminating the hen has been described by Burrows and Quinn (*20*). It consists in exposing the oviduct and injecting semen directly into the uterus. The hen is held with the left hand under her breast, the index finger between the legs, and the thumb

and other fingers around the legs. The loose skin of the abdomen is grasped with the tips of the fingers, thereby pulling the feathers away from the vent and forcing the abdominal contents into the smallest possible space. If properly done, this causes the vent to protrude slightly. The right hand is then placed above the vent so that the thumb and forefinger extend downward on each side, and the hen's tail is forced upward over her back by the heel of the right hand. When the hands are in this position, a sudden pressure between them will cause the oviduct to be everted as in normal mating. The eversion will be easier if the entire procedure is done quickly after the hen is picked up.

When the oviduct is sufficiently everted so that its orifice can be plainly seen, the inseminating syringe is introduced as far as it will slide easily, usually about 2 inches. Pressure on the abdomen is then fully released, while slight pressure is maintained on the syringe, which causes it to follow the retraction of the oviduct. When the oviduct is fully retracted, about one-third of the syringe should be covered. At this point the desired amount of semen is injected by gentle pressure on the plunger of the syringe. A common 1-cc. tuberculin-type syringe, graduated in 0.1-cc. units, is most satisfactory for this purpose. All equipment should be ready and the syringe filled with the requisite amount of semen before insemination is undertaken.

On account of its size, the turkey hen is handled somewhat differently. The operator picks up the hen with her head toward him, then stoops slightly and thrusts her head between his legs, which permits the bird to rest on his sloping legs (fig. 23, *C*). The legs are held in the right hand until the left hand is in position on the abdomen. From this point the procedure is similar to that for the chicken hen. To bring about eversion of the oviduct, considerable pressure must sometimes be applied; but if it is applied quickly, eversion will be brought about with less pressure than if it is applied slowly.

Using this method of insemination, workers have obtained 80-percent fertility in 31 turkey hens inseminated with 0.05 cc. of turkey semen at intervals of 1, 2, 3, and 4 weeks. These observations were based on a total of 948 eggs. In one special test mating of 10 turkey hens with an old tom, the percentage fertility was increased from 7.5 for natural mating to 88.4 when artificial insemination was used. No greater embryonic mortality was observed in eggs from hens artificially inseminated than in eggs from hens naturally mated. Hens that become fertile may retain their fertility for 3 to 4 weeks, but not all hens become fertile from a single insemination. If hens are properly inseminated with 0.5 cc. of viable semen twice at an interval of 3 to 4 days and this process is repeated every 3 weeks, 80- to 90-percent fertility should be obtained, although the degree of fertility varies with different hens.

In chickens, insemination with 0.1 cc. of good semen once each week should result in 80 to 80 percent fertility of the eggs, although fertility may vary somewhat from mating to mating. If the fertility for any mating under such procedure is low, it may be necessary to use more semen, to inseminate more frequently, or both. Ordinarily the best fertility is obtained with semen that has just been collected. Satisfactory methods have not yet been developed for storing semen more than a few hours.

As in normal matings, some hens, both chickens and turkeys, do not become fertile after careful and persistent inseminations. Such hens should not be kept in the breeding flock.

ARTIFICIAL INSEMINATION AN AID IN CONTROLLING VARIOUS DISEASES

Many diseases that attack the reproductive organs of animals may be spread by sexual intercourse; some are transmitted only in this way. Artificial insemination, if properly done, may prevent the spread of such diseases by breaking the link between male and female and between females through the male. If improperly practiced, however, it may spread disease. Most cooperatives obtain the services of a veterinarian or have access to veterinary advice for this work, which requires not only a knowledge of the symptoms of diseases but also cleanliness, sanitation, and the proper disinfection of utensils before and after use. In the prevention of disease, cleanliness of the artificial vagina is exceedingly important. Details on this point are given on page 7.

If artificial insemination is to play an important role in the control of disease, only healthy males should be used. Animals considered for this purpose should be given clinical and bacteriological examinations to determine whether they have any abnormal conditions that might interfere with fertility or lead to the transmission of infections. In connection with the clinical examination, semen samples may aid in determining the presence and location of infections and other disease conditions in the male genital tract, as demonstrated by Williams (*87*), Blom and Christensen (*13*), and Blom (*12*).

The danger of introducing diseases among cattle may be largely avoided by purchasing young virgin bulls. Cows to be used as dummies at bull studs should be obtained from herds with good breeding histories. As an added precaution, when making additions to or replacements in the herd, virgin heifers should be selected if practicable.

Diseases that are spread entirely or in part by sexual contact, and that can be controlled—at least to some extent—by artificial insemination, are discussed in the following paragraphs.

Dourine.—This is a disease of horses and members of the horse family caused by the protozoan parasite, *Trypanosoma equiperdum*. It is spread by contact of the membranes of the genital tracts during coitus. Through the use of the complement-fixation test, dourine has been eradicated from the United States, except for a few isolated localities in the Southwest, by the systematic elimination of all reacting animals. Artificial insemination prevents infection from spreading among clean stallions and mares.

Miscellaneous infections in mares.—A large percentage of all mares that conceive either abort or give birth to dead foals. Infections are a cause of part of this trouble. Varieties of streptococci, *Salmonella abortivo-equinus*, *Bacterium viscosum equi*, and viruses have been isolated from aborted fetuses. The mode of transmission of these infections is not always known, but in many instances the male may be involved. In established studs where the stallions and mares are

under constant supervision of a veterinarian, losses due to infectious organisms have been reduced to a negligible number. The importance of using disease-free stallions for artificial insemination cannot be overemphasized.

Coital exanthema (*vesicular venereal disease*, *genital pox*) *in horses and cattle*.—This is a highly transmissible, primarily venereal disease that rarely occurs in the United States but is common in Europe. It attacks the membranes of the genital tract and spreads to the skin bordering these regions. It is characterized by the appearance of vesicles that rupture to form ulcers. Affected individuals evidence considerable local pain. In cows the vulva becomes swollen, and the animal stands with an arched back and may bawl and urinate frequently. Owing to its short course of about 2 weeks and prompt healing, the effects on fertility are not outstanding. However, individuals, particularly bulls, may be left with extensive adhesions requiring surgical treatment to restore coital function. The effects of this infection on the uterus, germ cells, and fetus are not known. It is reported to be caused by a filtrable virus.

Bovine venereal trichomoniasis.—This disease is caused by *Trichomonas foetus* and is transmitted naturally only at coitus. Bartlett (*5*) reports that it can be transferred readily between females through careless use of instruments or to females through artificial insemination with semen from infected bulls. In females, the most consistent effect is early termination of pregnancy. In initial infections, cows return to estrum within 3 to 5 weeks after coitus. The infection generally continues for several months thereafter, during which time sterility persists. In bulls, the organism, which ordinarily lives on the surface of the preputial membranes and glans penis, produces no significant symptoms and ordinarily influences neither fertility nor potency. Diagnosis is based on actual demonstration of the protozoan, *Trichomonas foetus*, in material collected from the genitalia of females or bulls. Pierce (*61*) reports the presence of agglutinins in the vaginal mucous secretions of infected cows and has developed a method that is accurate in detecting herd infection but does not detect all infected individuals in a herd.

Bartlett (*6*) reports that since there is no treatment capable of interrupting the course of infection in the female, because of the self-limiting nature of this disease, it can be eradicated by systematic controlled breeding practices. Subsequent to (1) completion of a normal pregnancy, (2) passing of two estrus periods, and (3) resting at least 90 days after parturition, previously infected females may be safely permitted coitus at their third or subsequent estrus periods with uninfected bulls. Artificial insemination as an adjunct in a hygienic breeding program has been found to be effective in eliminating *Trichomonas foetus* infection from affected herds. Trichomonad-infected bulls have no use as sires. Recently, encouraging results have been obtained in treating infected bulls for this disease.

Bovine brucellosis.—The problems incident to the control and eradication of this disease are well known. Occasionally the organism localizes in the genital tract of the bull and has been isolated from the semen of such individuals. There is circumstantial evidence that cows have been infected by artificial insemination with semen from infected bulls as reported by Bendixen and Blom (*7*). The occurrence

of brucellosis-infected bulls is rare; thus the use of artificial insemination would be a minor preventive measure in the spread of the disease.

Granular vaginitis (nodular venereal disease) in cattle.—This condition is so common that some animals with lesions characteristic of it can be found in most herds of cattle. In females the lesions may be confined to the vulva, occurring only around the clitoris. In others, the vulva and vagina may exhibit moderate to severe inflammation with a rather general distribution of nodules. Sexually immature heifers often have lesions. It is not unusual to find severe lesions in females in all stages of pregnancy, whether their prior breeding records were normal or abnormal. Characteristic nodules and inflammation may occur on the preputial membrane and glans penis of bulls. Laboratory studies have not revealed that a specific infectious agent is consistently present in affected individuals. It is possible that this condition is a symptom and not primarily infectious.

Severely affected animals may be benefited by occasional douches with mild antiseptic solutions or by occasional insufflation with absorbent antiseptic powders. Too vigorous or frequently repeated treatments are harmful.

Through artificial insemination it was hoped to increase fertility in affected females by placing the semen beyond the lesions in the vagina. Results have not shown that such a practice is beneficial. Artificial insemination with semen from a nonaffected bull should eliminate the bull as a possible source of granular vaginitis.

Vibrio fetus (vibrionic abortion) in cattle and sheep.—The bacterial organism, *Vibrio fetus*, is a cause of occasional abortions throughout the United States. In cattle pregnancies may be terminated at almost any stage, but most frequently between the fourth and sixth months. The manner of transmission is unknown although Stegenga and Terpstra (*79*) have reported its isolation from a bull. Both American and European observers have stated that a lowered conception rate occurs in infected herds. However, there are many authentic reports (mostly verbal) that infected animals experience no delay in conception.

Plastridge and Williams (*62*) reported the use of the agglutination test for the diagnosis of this disease in cattle. However, this test has not proved entirely satisfactory. Stegenga and Terpstra (*79*) describe a method of testing for *Vibro fetus* by using the mucous secretions of the vagina. Its presence can be established by microscopic or cultural demonstration of the organism from an aborted fetus. Since little is known concerning the manner of transmission and the methods of diagnosis are difficult, specific measures for coping with this disease in affected herds are not available. All females showing signs of abortion and all those at normal parturition should be isolated and quarantined until genital discharges terminate. The use of semen from clean bulls would eliminate the male as a possible source of spread of infection.

Ovine balanoposthitis (inflammation of the glans penis and prepuce).—Tunnicliff and Matisheck (*83*) report the isolation of a virus as the causative agent of this disease. Secondary invasion of bacteria adds to the severity of the lesions, which are characterized by formation of ulcers and scabs. The genital organs of both ewes and rams are the site of infection. It is reported to be spread at the time of

coition. Artificial insemination should play an important part in controlling its spread and preventing infection of valuable rams.

Swine brucellosis.—Manthei (*45*) states that this disease is commonly transmitted from boar to sow and vice versa when breeding. He exposed susceptible sexually mature boars to sows that were persistently eliminating *Brucella suis* from the vagina. Genital infection was definitely established in 3 of 5 boars exposed by breeding only and in the 2 remaining boars from teasing only. Of 14 clean sows bred to boars secreting *Br. suis* in their semen, infection was later demonstrated in all but 1 animal. Crawford and Manthei (*22*) suggest only two methods that offer a solution to *Br. suis* infection in swine: (1) eradicate the entire herd, or (2) retain weanlings negative to the blood test and dispose of the remainder of the herd. Artificial insemination would prevent valuable boars from becoming exposed through coition.

Vent gleet (*cloacitis*) *in chickens.*—Hall and Wehr (*28*) describe the symptoms of this disease as reddening and swelling of the skin around the vent accompanied by ulceration and usually offensive odor. The causative agent is not known. The site of infection indicates that it can be spread through coition. Artificial insemination would prevent exposure of valuable males.

Miscellaneous infections.—In animals of either sex, infections may ascend the urogenital tract or enter the reproductive organs from the blood stream. The female is exposed at the time of parturition, especially if the fetal membranes are retained. Many organisms, including streptococci, staphylococci, corynebacteria, diplococci, molds, and intestinal organisms, have been cultured from aborted fetuses. These organisms are found in the surroundings of animals and may be present in the vagina of the female and sheath of the male. It has not been determined whether all of these are capable of producing infection in the genital tract. Neither has it been determined whether semen containing these organisms or other organisms, such as a virus, if introduced into the uterus of a cow would be capable of producing infertility in the female, although it is very probable that this could occur. Clinical examination of the genital tract together with laboratory examinations of the semen will reveal, in most instances, whether or not localized infections are established in the male reproductive organs. Bacterial infection of the female reproductive tract may be influenced by the site of semen deposition.

Fetuses may die and remain in the reproductive tract as mummies. The cause of death is not known. The fetuses have been found sterile for bacteria and virus.

A careful check should be made of the breeding history of the herd from which males that have been used for natural breeding are to be selected. The shy breeder is a difficult problem in artificial insemination. There is no known cause for this condition. One of the causes may be an unknown venereally transmitted agent. If so, the number of females requiring repeated services would be a valuable index in selecting a male. In cattle herds, for instance, in which 20 percent or more of the females, including virgin heifers, require an average of four or more services per conception, the bulls may be carrying an infectious agent.

TRAINING OF TECHNICIANS

The use of artificial insemination has expanded so rapidly that an adequate number of veterinarians with professional training in sanitation and disease control have not been available to act as inseminators; consequently, it has been necessary to train technicians for this work. These technicians need instruction in methods of cleaning and sterilizing their equipment and in sanitary procedures to be observed within a herd as well as between herds. They should be informed on the bacterial flora of semen (*24*, *27*, *53*), collected in the artificial vagina and also on the benefits of refrigeration in keeping the bacterial count as low as possible. Courses of training for technicians are offered by many of the larger artificial insemination cooperatives and associations. These organizations generally have a qualified veterinarian to give specific training.

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